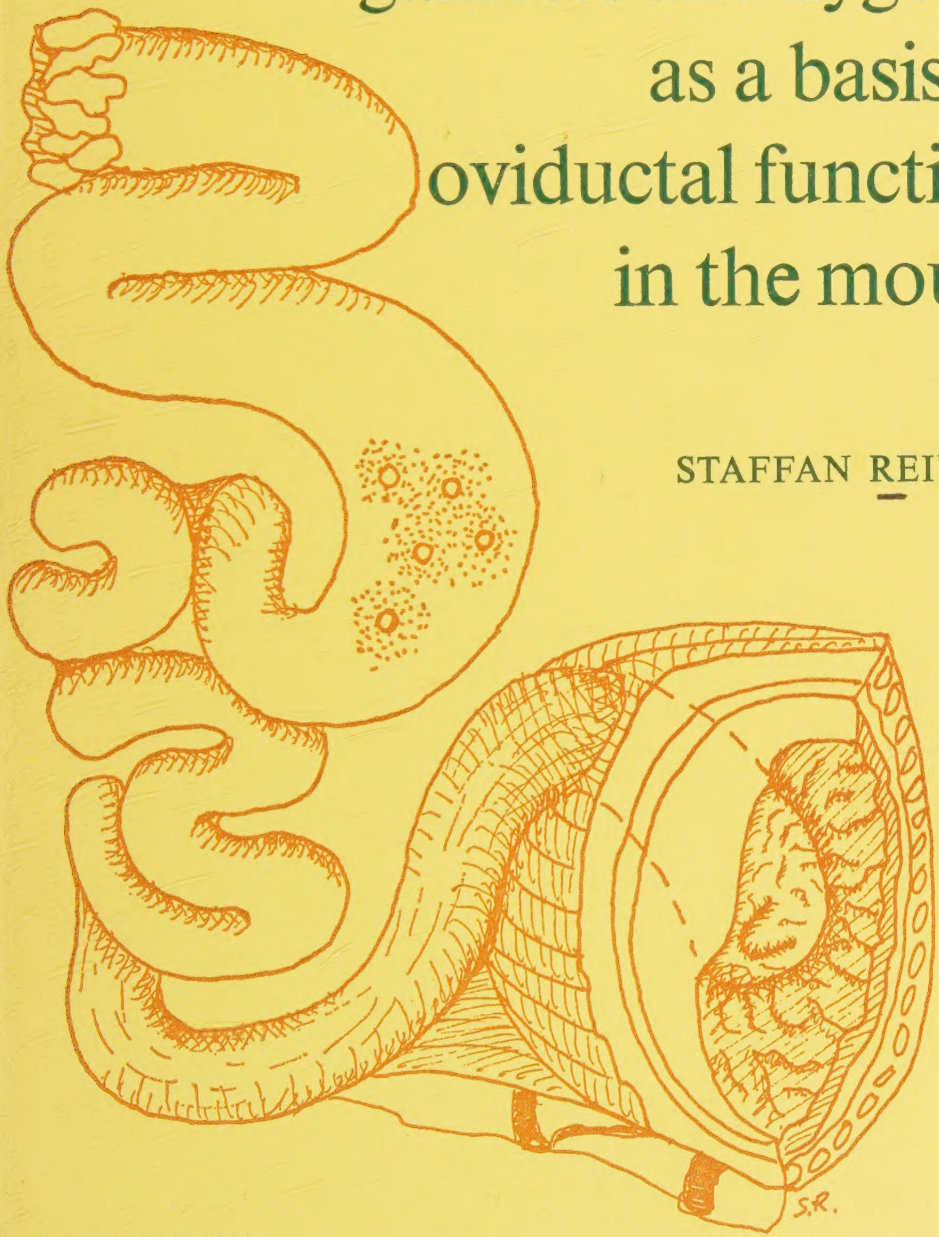
Morphology of oviduct gametes and zygotes as a basis of oviductal function in the mouse

by

STAFFAN REINIUS



UPPSALA 1969

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PREFACE

This report presents the morphology of the mouse oviduct with gametes and zygotes *in situ*. Since the method used preserves almost completely the structural interrelationship of the living animals, new aspects have been obtained on the epithelial secretion, the sperm cell migration, and the development and transport of the ovum and zygote. The study was performed at the Institute of Human Anatomy, Uppsala, Sweden.

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INTRODUCTION

In the mouse and various other mammals, the ova and zygotes (cleavaging stages) remain in the oviduct for about three days. During the first day the spermatozoa and the ova pass to the ampulla, where fertilization takes place. On the second day the zygotes (at the 2-cell stage) lie in the isthmus. At the end of the third day they pass through the last segment of the oviduct called the junctura and are now at the 5-to 8-cell stage.

During this time several important processes occur within the oviduct, which presumably place specific demands on its structure both as a secretory organ and as an organ of transport and/or conduction.

Previous techniques have not allowed detailed studies of the structure and function of the oviduct in the segments where the gametes or zygotes are lying. Therefore, the secretory activity of the oviductal epithelium at the passage of gametes and zygotes is essentially unknown. Similarly the structural relationships between the gametes or zygotes and oviductal epithelium influencing their passage, require a closer study.

The working hypothesis in the present study was the question of whether that the composition of the secretion differs in the various segments of the oviduct. This seems reasonable, since it is known that the requirements of the ova/zygotes vary at different developmental stages and locations. Further, the lumen and the epithelium of the oviduct are expected to have structural features adapted to the passage of the gametes and zygotes.

The new preparatory technique of aortic glutaraldehyde perfusion and Epon embedding leaves the relation between the gametes or zygotes and the oviductal epithelium almost intact. The mouse has been suitable for its small size and easy handling in reproduction. Thus, information on the differential composition of the secretion in the different segments can be obtained and the mechanism of sperm cell migration and of the ova/zygote transport can be studied to advantage.

The study consists of four parts, the first deals with the ultrastructure of different segments of the oviductal epithelium and gives new information about the nature of the secretion. The second deals with the

structural relation between the spermatozoa and the oviductal epithelium, particularly at the uterine oviduct opening and the junctura, with special consideration of possible mechanisms that make the spermatozoa enter and pass through the oviduct. The third part deals with the structural relations between the ova/zygotes and the oviductal epithelium and the possible mechanisms that influence the transport. The last part,finally, deals with the ultrastructure of the ova/zygotes and their relation to the oviductal epithelium and elucidates those metabolic activities of the ova/zygotes which have significance for evaluation of the segmental oviductal secretion.

I. SECRETORY ACTIVITY OF OVIDUCTAL EPITHELIUM

The oviductal secretion contains substances which are necessary in the final maturation of the spermatozoa and the ova and for the fertilization process (cf. Hafez 1967 and Restall 1967). The secretion also provides a transport vehicle and nutrients for developing zygotes. The secretory activity of the oviduct may hence be considered in relation to the gametes and the development of the zygotes. Segmental differences can be expected, since the ova/zygotes require different environmental conditions during their early development, as indicated by *in vitro* studies (Biggers et al. 1967, Whittingham 1968a, Brinster 1969). Even epithelial activities related to the ova/zygotes may be expected (cf. Fredricsson 1969).

The method used for evaluating the composition of the oviduct secretion are mainly biochemical and histological (cf. Bishop 1961, 1969). Biochemical studies have as yet only been performed on comparatively large animals, e.g. the rabbit, and have the disadvantages that they need long periods for collection, and do not distinguish between different segments of the oviduct (for references see Hamner and Fox 1969).

Conventional histological techniques are hampered by the relatively low magnification used and the possibilities of creating artifacts; histochemistry has given information on products and enzymes in the epithelium and in the secretion, but is restricted to the resolving power of the light microscope (cf. Fredricsson 1969). Although electron microscopy has given information about the secretory activity of the oviductal epithelium no previous study has been performed showing the functional stages of gametes and zygotes in their passage along different segments of the oviduct (rat: Nilsson 1957, Borell et al. 1959, Wolf 1960; rabbit: Borell et al. 1956, Nilsson 1958, Hashimoto et al. 1959a,b, Moricard and Palmer 1960, Nilsson and Rutberg 1960; man: Moricard and Palmer 1960, Vokaer and Vanderbeken 1960, Björkman and Fredricsson 1962, Fredricsson and Björkman 1962, Hashimoto et al. 1962, 1964, Stegner 1962, Shimoyama 1965, Clyman 1966).

A study of the segmental and local composition of oviductal secretion

at the functional stages requires a technique that maintains the position of the gametes and zygotes and makes possible local or segmental sampling of the secretion. With the improved morphological techniques of glutaraldehyde perfusion fixation and plastic embedding, it is now possible to study whole female genital organs of small laboratory animals with gametes and zygotes preserved *in situ* (Reinius 1965, 1966, 1967a,b, Nilsson and Reinius 1969).

The aim of this study was to establish whether different local or segmental epithelial secretions occur in the segments where the gametes or zygotes are present. Therefore, structure of the oviductal epithelium in the mouse was studied by light and electron microscopy in the different segments, and was related to the different functional stages of sperm cell migration and to the transport and development of ova/zygotes.

MATERIAL AND METHODS

Virgin females (aged 12 to 25 weeks) and males (aged 12 to 52 weeks) of the CBA mouse strain were used. They were kept separately from other animals in a room and conditioned to artificial light from 05 to 19 hr. The experiments were continued throughout the year. The food was composed of standard rodent pellets and corn pellets. For mating, two females were left with one male, either overnight (about 16 to 09 hr) or for short periods during the night. Pregnancy was determined by the findings of a vaginal plug. Day 1 was the day when the vaginal plug was found. The animals were fixed for studies of the following functional stages at the times given: sperm cell migration at Day 0/22 hr to Day 1/05 hr, unfertilized ova lying in the ampulla at Day 1/05 to 08 hr, pronuclear stages of the ovum lying in the ampulla at Day 1/10 to 17 hr, 2-cell zygotes lying in the isthmus at Day 2/08 to 17 hr, and 5- to 8-cell zygotes in the junctura at Day 3/08 to 20 hr. See Table I.

Fixation was performed by aortic perfusion with a solution of 2.5% glutaraldehyde in a Sørensen phosphate buffer, pH 7.3, osmolality about 450 mOsm, as determined by freezing-point depression. Postfixation was performed in 1% osmium tetroxide in the Sørensen phosphate buffer, pH 7.3. The fixatives were used at room temperature.

The perfusion aggregate consisted of a flask filled with the fixative under an excess gas pressure of about 0.2 to 0.3 atmospheres from a nitrogen cylinder. An administration set with a cannula for intravenous

Table I.

Time of Pairing	Time of Fixation Day/Hr	Hrs after Copulation	Hrs after Sperm Penetration	Stage of Development	Location of Ova/ Zygotes	No. of Animals
21-21.15**	0/22	3/4	-	I Spindle	Ovary	1
21-22.30**	1/00	2 1/2	-	II "	"	1
21-23hr	"	1-3	-	I "	"	2
22-01hr	1/01	0-3	-	I "	"	1
23-04hr	1/05	5*	-	II "	Ampulla	4
16-08hr	1/08	8*	-	II "	Preampulla (one ovum) and Ampulla	2
Total number of animals studied for sperm cell migration and/or unfertilized ova in the preampulla and ampulla						11
16-09hr	1/10	10*	4*	Pronucleus	Ampulla	5
"	1/15	15*	9*	"	"	2
"	1/16	16*	10*	"	"	5
"	1/17	17*	11*	"	"	4
"	1/19	19*	13*	"	"	2
Total number of animals having pronuclear ova in the ampulla						18
16-09hr	2/08	32*	26*	2-cell	Isthmus	1
"	2/09	33*	27*	"	"	2
"	2/10	34*	28*	"	"	6
"	2/15	39*	33*	"	"	3
"	2/16	40*	34*	"	"	6
"	2/17	41*	35*	"	"	5
Total number of animals having 2-cell zygotes in the isthmus						23
16-09hr	2/10	43*	37*	3-cell	Isthmus	1
16-09hr	3/08	56*	50*	5-8-cell	Junctura	2
"	3/10	58*	52*	"	"	2
"	3/16	64*	58*	"	"	2
"	3/17	65*	59*	"	"	9
"	3/20	68*	62*	"	"	1
Total number of animals having 5-8-cell zygotes in the junctura						16
					Total	69

* Time estimated

** The later time refers to the time of observed copulation

infusion was connected to the perfusion aggregate.

The perfusion was peformed as follows. The animal was anaesthetized by an i.p. injection of 0.8 cc 0.1% Nembutal (Abbott) in distilled water and then nailed to a cork plate. The abdomen was opened by a midline incision with lateral extensions, the intestines were retracted to the left side, and the abdominal aorta was exposed in the retroperitoneal space from the level of the renal arteries to a few mm caudally. The uterine cornua, oviducts and ovaries were usually protected by intestines and fat, and did not come into contact with the air or the instruments. The aorta was clamped just below the renal arteries and the cannula of the perfusion aggregate was quickly inserted into the aorta just caudal to the clamping point. Before injection of the fixative, the vena cava was cut in order to facilitate the escape of the blood and the fixative through the venous side. The fixative reached the genital organs through the uterine arteries and left it through the uterine and the ovarian veins. The perfusion was continued for about five minutes. From the sequences of events at the perfusion procedure the rapidity of fixation was estimated. The fixative emptied the vessels of the genital organ within 10 sec after the aorta was clamped. After 30 to 60 sec the tissues had hardened and turned yellow, indicating completed fixation (cf. Maunsbach 1966a and Hopwood 1967).

Fixation was also performed on Nembutal anaesthetized and opened animals by pouring the glutaraldehyde into the abdomen.

Dissection. In the next preparation step the genital organs were gently dissected free as an entirety, avoiding pinching and tearing of the organs. The dissection was facilitated by the stiffnes of the organs. The specimen was then immediately placed in a vial containing the fixative and stored for a period varying from a few hours up to some days at a temperature of about 4°C.

The top of the uterine horns with the oviducts and ovaries was then cut off and carefully freed from excess adipose tissue. The ovarian bursa usually opened at this procedure. The specimen from one side was then directly postfixed without further dissections, whereas that from the other side was dissected further in a stereomicroscope. The oviduct was separated from the ovary and divided into four parts: the preampulla constituted loop 1; the ampulla loop 2; the isthmus loops 3 to 7 or 8; and the junctura constituted the last one, loop 8 or 9, and the intramural part of

the oviduct, which was connected to the top of the uterine horn. The different loops of the isthmus had been studied separately in a few specimens in order to elucidate further subdivisions of this segment.

Postfixation was made for 3 to 5 hr in osmium tetroxide, sometimes after rinsing in the buffer. The embedding was performed in Epon. The specimens were orientated in the capsules in a suitable position for each segment before polymerisation.

Sectioning for light microscopy of the plastic embedded specimens was performed on a Porter Blum microtome or an LKB Ultrotome, using a dry method without a trough (Reinius 1966). Five to ten μ thick sections were cut and successively discarded until a representative part of either a longitudinal or a cross section of the oviduct epithelium appeared or ova/zygotes were observed in the stereomicroscope of the microtome. Then 2 to 4 μ thick sections, with an area of up to 4 x 7 mm, were placed on slides and stained in 1% Toluidine blue in a 1% borax or a 1 to 2% sodium bicarbonate solution (Pease 1964, Reinius 1966). Often the undissected specimen from the other side of the animal was also sectioned. Sectioning of oviduct segments at the time of sperm cell migration was performed only on those specimens that had spermatozoa in the uterus.

The ova/zygotes could be distinguished in the unstained sections lying on the knife (cf. Reinius 1966). In the ampulla, the ova were seen to be surrounded by the follicle cells. In the isthmus, only such loops were distended where the zygotes were situated; the latter then appeared as dense round spots of appropriate sizes; in the junctura, the zygotes appeared as less dense spherical spots in the darkly-stained epithelium. After fixation by pouring, on the other hand, the junctural zygotes could be observed only after staining serial sections, which makes this fixation a much more uncertain and time-consuming method when looking for zygotes.

Sectioning for electron microscopy was performed on an LKB Ultrotome after the specimens had been trimmed to a maximum area of about 0.5 mm^2. The sections were picked up on one-hole grids according to Galey and Nilsson (1966) and contrasted with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963). An RCA EMU 3B microscope was used.

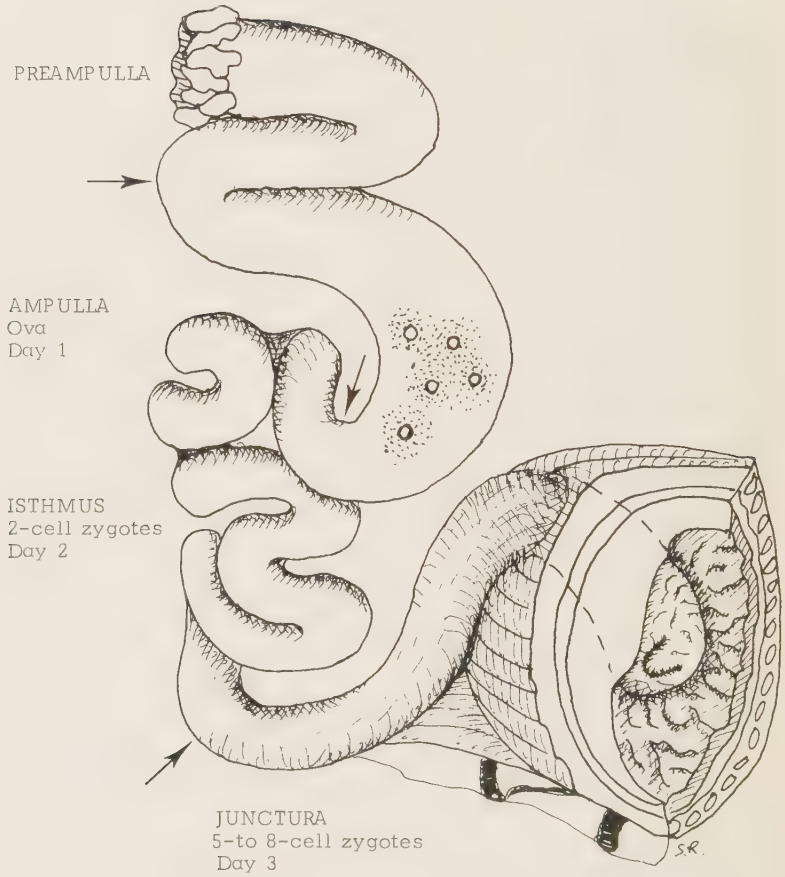

Fig. 1. Schematic drawing of the mouse oviduct showing the divisions of the oviduct and the stage and location of the ova/zygotes in the different segments. Note the intramural part of the junctura and the semilunar uterine opening of the oviduct at the colliculus tubarius.

RESULTS

The mouse oviduct is coiled, forming about eight loops. It is connected to the ovary by the almost completely closed bursa ovarica. The last portion of the oviduct runs intramurally in the uterine cornu and ends with the colliculus tubarius (Fig.1). The oviduct was divided into four segments: 1) the preampulla, 2) the ampulla, 3) the isthmus, and 4) the junctura.

The conditions at the time of ovum/zygote passage in the four segments are described first below. Structural changes at other stages of the passage or at the sperm cell migration are then reported.

Preampulla

The preampulla comprised the fimbriae and first loop. The muscular tissue consisted of only two or three layers of cells, mostly arranged longitudinally. The propria was scanty and extended into high longitudinal folds of epithelium. The folds met in the middle, thereby appreciably restricting the free space in the lumen. On one occasion an ovum was observed in the preampulla (cf. Reinius 1969b).

The epithelium contained mostly ciliated cells (Fig.3); nonciliated cells, appearing singly or in groups of a few, were dispersed among the ciliated ones in an approximate ratio of 1:5-10.

The ciliated cells (10 to 15 μ in length) had regular microvilli (2.0 μ), and regular cilia (2.5 to 3.0 μ)(Fig.4). The nucleus was almost rectangular in shape. The nuclear membrane had only a few indentations. The mitochondria were rod-like, and a few of them appeared branched. The Golgi complex was small and excentrally placed apical to the nucleus. The endoplasmic reticulum was poorly developed and consisted of a few scattered, short and rough tubules. Free ribosomes were numerous. Often a large lipid droplet and/or vacuole was observed, the latter sometimes occupying the whole width of the cell (Fig.3). Both the lipid droplets and the vacuoles were more frequent in the ciliated cells at the ovarian end of the preampulla, and could be found either in the apical or basal part of the nucleus.

The nonciliated cells were 15 to 17 μ high. The apical part of the cell was broader than the basal part and projected or bulged above the surface of the ciliated cells (Fig.3). The surface of the nonciliated cell possessed a few short, irregularly distributed microvilli. The nucleus was large, often extending into the bulging apical part of the cell, and had

few indentations. The cytoplasm stained darker than that of the ciliated cell. The mitochondria were small and oval or rod-shaped. The Golgi complex was rather large, extending over the nucleus. The endoplasmic reticulum was moderately developed and consisted mostly of long parallel granular membranes; wider cisternae were sometimes observed. The cisternae were filled with a moderately electron dense material. Occasional dense granules, about 0.4 μ in diameter, were situated apically.

No structural changes of the epithelium were observed during Days 1 to 3.

Ampulla

The ampulla constituted the second loop and was easily recognized at Day 1, by its distension with fluid and ova.

The outer diameter of the distended ampulla was about 500 μ; the epithelium formed small and sparse longitudinal folds into the wide lumen.

The epithelium consisted mainly of nonciliated cells, but ciliated cells were rather frequent and they were relatively more numerous towards the preampulla (Fig.5). Intracellular vesicles provided with microvilli (Fredricsson and Björkman 1962) and sometimes a few cilia were occasionally seen in both types of cells. The ciliated cells had the same structure as in the preampulla, although they had only few lipid droplets and vacuoles.

The nonciliated cells were low (10 to 12 μ), cuboidal, and had only slightly bulging apical parts (Fig.5). The surface of the nonciliated cell possessed relatively few microvilli, 0.5 to 0.7 μ in length (Fig.6). The nucleus lay centrally in the cell, was round or oval, and devoid of indentations. The mitochondria were small and oval or rod-like. The Golgi complex was large and consisted of many flattened cisternae and vesicles in the apical cytoplasm. The endoplasmic reticulum was extensive and formed cisternae of irregular width and vesicles. The membranes had some ribosomes attached to their distended parts. The cisternae and vesicles were filled with a moderately electron dense substance. Electron dense granules were much more numerous in the nonciliated cell of the ampulla than in corresponding cells in the preampulla. They were about 0.4 μ in diameter and principally of an apical localization in the cell. No local structural differences related to the position of the ova were observed.

Structural changes were observed during the functional stages at the time of sperm cell migration before ovulation and when zygotes lay in the

isthmus and the junctura. At these stages no marked demarcation against the preampulla or the isthmus were seen. The undistended ampulla measured 300 μ in diameter, and the epithelial folds were high, meeting each other in a narrow lumen. The ciliated and nonciliated cells were higher (15 to 20 μ), and the nonciliated cells had a more bulging apical part, which, however, was not much wider than the basal part (Fig.7). The endoplasmic reticulum of the nonciliated cells constituted more and longer tubules and dilated cisternae, and the dense granules were more numerous, than when the zygotes were present in the ampulla. This was most pronounced at the time of sperm cell migration, before ovulation Day 0/22 to 01/01 hr and before the distension of the ampulla.

Ampullary - isthmic junction

An ampullary-isthmic junction was formed by the narrowing of the oviduct loop next to the ampulla. When the ampulla was distended, the outer curve of the loop showed only a gradual thickening of the muscular wall, but its inner curve formed a massive thickening of mainly circularly arranged muscle cell like a muscular sphincter. The opening into the isthmus was closed by long mucosal folds, filling its lumen and protruding into the ampullary lumen. The epithelium of these folds consisted mainly of nonciliated cells. At other stages the ampulla was not distended and the lumen still closed, as also were the ampulla and neighbouring isthmic loops (cf. Reinius 1969b).

Isthmus

The isthmus formed a long and coiled part of the oviduct, constituting the 3rd to the 7th or 8th loop. The muscular layer had a mainly circular orientation and measured 20 to 30 μ. Longitudinal epithelial folds were seen only on the loop next to the ampulla. Low circular folds appeared in the rest of the isthmus (Fig.8). The lumen of the loops where the zygotes at the 2-cell stage lay, was distended; other loops were closed. The zygotes were often grouped together and lay not more than two loops apart (Fig.8).

Ciliated cells were sparse and were located in epithelial grooves.

Nonciliated cells were of two kinds, according to their ultrastructure. One was similar to those in the ampulla, though higher, and was most abundant in the first and second loops of the isthmus.

The other nonciliated cell type was predominant and could be regarded as the characteristic isthmus cell (Figs.7 and 8). This nonciliated cell

was 10 to 30 μ high and had 0.8 to 1.2 μ long relatively regular microvilli. The nucleus was centrally located and ovoid, and the nuclear membrane had few indentations. The mitochondria were oval to rod-shaped and some were branched. They were located mainly in the apical cytoplasm. The Golgi complex was well developed. The main part of the endoplasmic reticulum was granular and was usually located below the nucleus in stacks of many parallel tubules. Also an agranular endoplasmic reticulum was seen in this isthmus cell, mainly in the apical part. Dense granules were rarely seen. Glycogen granules, mostly of the β-type (Drochmans 1962), were frequent and sometimes formed large aggregates. They often were closely associated with the agranular endoplasmic reticulum. Vesicles provided with microvilli and sometimes a few cilia were occasionally seen. No local structural differences related to the position of the zygotes were observed, except for the closed lumina in the loops devoid of zygotes.

The lumen was normally closed when the zygotes were not in the segment. Solitary sperm cells were rarely seen either in wide or narrow loops. The only obvious morphological change of the epithelium was a change in the number of glycogen granules in the typical isthmus cell. They were usually absent when the zygotes lay in the junctura and reappearing to a smaller extent during transport of spermatozoa on Day 1.

Junctura

The junctura constituted the last loop of the extramural oviduct and the intramural oviduct with the colliculus tubarius. The external part rested on the top of the uterus and curved steeply into the anti-mesometrial side of the uterine cornu. The intramural part ran obliquely in the uterine wall and ended on the colliculus tubarius.

The muscular layer was thicker than in the isthmus. It consisted of mostly circularly arranged cells. The intramural part of the muscle layer diminished towards the top of the colliculus. A thin external layer of longitudinally running muscle cells had direct continuity with the external uterine muscle layer. The muscular layer was separated from the epithelium only by two or three layers of connective tissue cells of the lamina propria. The fibrous tissue and the epithelium formed a framework of moderately high longitudinal folds along the junctura, except in the last portion at the top of the colliculus, where folds were lacking. The folds of the uterine epithelium lay close to the outside of the colliculus.

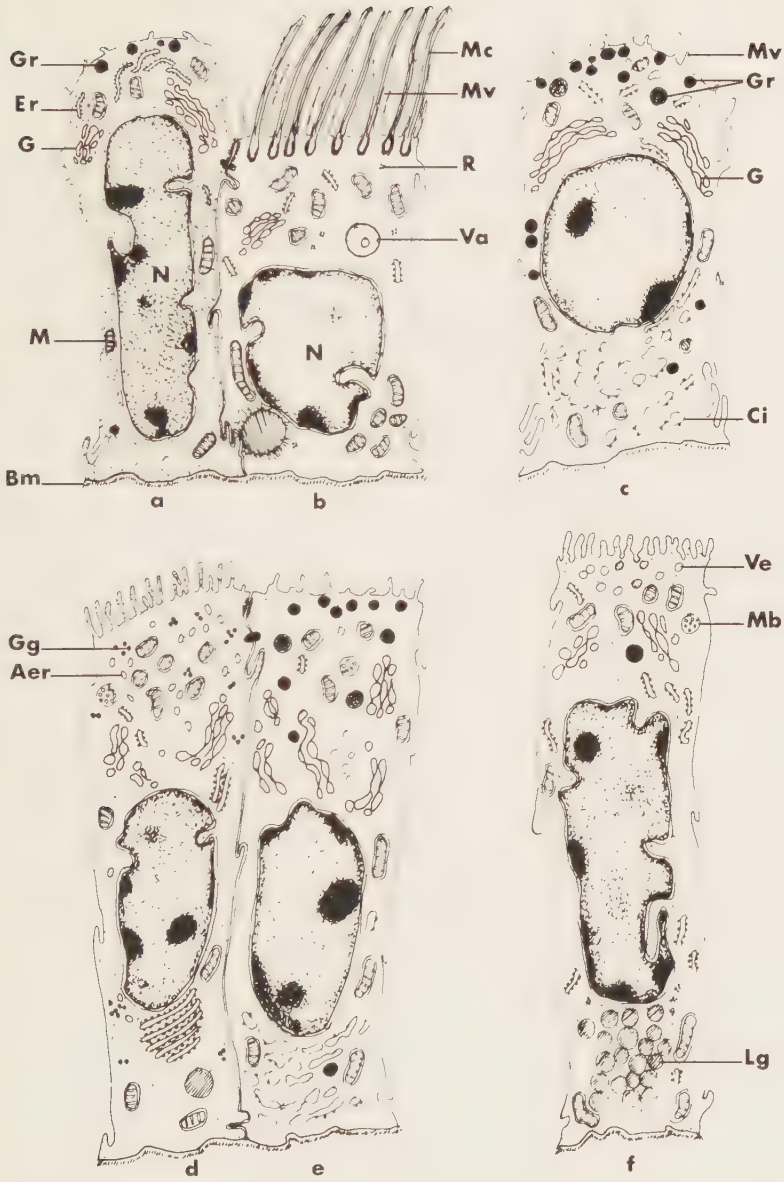

Fig. 2. Schematic drawing of the different epithelial cell types in the oviduct of the mouse. a) preampullar nonciliated cell, b) ciliated cell; c) ampullar nonciliated cell; d) and e) isthmic nonciliated cells; f) junctural nonciliated cell. Agranular endoplasmic reticulum (Aer); basal membrane (Bm); dilated cisternae of the endoplasmic reticulum (Ci); endoplasmic reticulum (Er); Golgi complex (G); glycogen granules (Gg); granules (Gr); lipid granule (Lg); mitochondrion (M); motile cilium (Mc); multivesicular body (Mb); microvillus (Mv); nucleus (N); ribosomes (R); vacuole (Va); vesicle (Ve).

The lumen of the junctura was narrow after preservation by perfusion fixation. Usually the epithelial surfaces lay in close contact, but spaces of only a few microns were sometimes formed. Where the zygotes (5- to 8-cell stage) lay, the epithelium followed the outline of the almost spherical zygotes, leaving no or very little space for fluid. After fixation by pouring, on the other hand, the lumina of the uterine end of the junctura and of the uterus were notably widened (see Figs. 11 and 12).

Ciliated cells did not occur in the junctura.

The nonciliated cells had a characteristic appearance. Their height was about 12 to 35 μ. Their surface possessed a regular border of microvilli (about 0.5 μ in length) and presumably pinocytotic invaginations (Fig.16). The elongated nucleus occupied the middle third of the cell. The nuclear membrane formed several deep, irregular indentations. The cytoplasm was rather dense. The mitochondria were oval or rod-shaped and mostly situated apically. The Golgi complex was large; it consisted of several stacks of cisternae and associated vesicles and was located supranuclearly. The granulated tubules of the endoplasmic reticulum were scattered in the cytoplasm. Small smooth vesicles (0.1 to 0.2 μ) and coated vesicles (about 700Å) were present in the apical cytoplasm. Some apical vesicles sometimes contained irregular dense material. Dense bodies and multivesicular bodies were fairly numerous in the apical cytoplasm. Lipid droplets with a diameter of 0.4 to 0.5 μ were numerous in the basal part of the cell. Structural differences, such as variations in the number of apical vesicles, were observed, but these could not be related to the position of the zygotes.

Morphological changes were observed during Day 1 and 2. The cells were lower, 15 to 25 μ, and the cytoplasm was less electron dense. The nucleus was oval and almost devoid of indentations. On sperm cell migration, early Day 1, the number of multivesicular bodies was increased, and glycogen granules often arranged in aggregates were numerous in the cytoplasm (Fig.15). Late Day 1 and Day 2 the microvilli, the plasma membrane in vaginations, the apical vesicles, and the small coated vesicles were fewer than when spermatozoa or zygotes were present.

DISCUSSION

Several requirements have to be fulfilled before the different secretory activities of the oviductal epithelium can be elucidated by a morphologi-

cal study. The epithelial cells of the tract have to be adequately preserved and the luminal width and the relation of the ova/zygotes to the epithelium must be retained. Without these minimum requirements the findings would be of limited value. Ordinary techniques for electron microscopy, however, include manipulation and dissection of the organ, resulting in lost structural relationships.

The development of perfusion fixation with aldehydes, preferably glutaraldehyde, with postfixation in osmium tetroxide, have given quite new tools for fixation of the tissues in a state which is similar to that of the living animal. A variety of tissues, including the female genital organs, are best preserved by glutaraldehyde in a concentration of about 2.5%, an osmolality about 450 mOsm, a neutral pH and a good buffer system such as the Sørensen phosphate buffer; the hydrostatic pressure at the perfusion should correspond approximately to the blood pressure of the animal (Christensen 1965, Fahimi and Drochmans 1965a,b, Reinius 1965, 1967a,b, Rostgaard and Behnke 1965, Schultz and Karlsson 1965, Ericsson 1966, Maunsbach 1966a,b, Fahimi 1967, Forsmann et al. 1967, and others).

The advantages of perfusion fixation lie in the rapidity of the process and the uniform preservation of the entire organ, which is fixed in situ and without preceding manipulation; the hardening of the tissues by the fixative further facilitates dissection of the organ (Maunsbach 1966a, Forsmann et al. 1967, and the present findings).

The extent to which these requirements of the experiment are met will be discussed regarding the ultrastructure of the epithelium, the luminal width of the oviduct and the position of the ova/zygotes.

The epithelial cells showed no specific autolytic artifacts similar to those which have been reported to appear within a few minutes after the blood supply has been cut off in a variety of tissues (Nilsson 1959, Sjöstrand 1967, Wischnitzer 1967). Dilated endoplasmic cisternae were seen in the ampullary nonciliated cell. However, since the cisternae were filled with an electron dense material, the swollen state was probably not an artifact.

Preservation of the width of the tubal lumen is also of particular interest. Pronounced shrinkage or swelling of the surrounding tissue as effects of fixation and embedding, as well as contraction of the muscular tissue as an effect of the glutaraldehyde, can be excluded in the pre-

sent study, since the lumina of the various segments have similar appearances both after perfusion fixation and after freezing with carbonic-acid snow and cryostat sectioning (Reinius, unpublished). Further, the dilatation of the ampulla and the isthmic loops containing ova/zygotes observed after perfusion fixation and freezing have also been observed on living oviducts *in situ* (Lewis and Wright 1935, Alden 1942, Blandau 1969, and Reinius, unpublished). General anaesthesia with barbiturates is known to influence the activity of the autonomic nervous system and thus may influence the contraction of the muscular wall and the width of the lumen. However, the anaesthesia seemed to have had no significant effect in the present study, since the luminal width and the location of ova/zygotes showed constant results at similar stages, after decapitation of the animal and freezing (Reinius, unpublished).

The relation between the ova/zygotes and the epithelium is probably left intact during the preparation, when they are lying in the narrow passages in the preampulla and the junctura, since the ova/zygotes seem to be captured by the epithelial walls (cf. Reinius 1969b). When lying in the wide luminal spaces in the ampulla and the isthmus they are freely movable, and their initial position probably changes during the preparation. Since the position of the zygotes in these segments probably alters rapidly *in vivo* (Burdick et al. 1942, Humphrey 1968), local structural differences within the dilated parts will not have any significance for the evaluation of local interaction between the zygotes and the epithelium.

The *oviductal epithelium* contains two main types of cells - the ciliated and the nonciliated. The distribution of these cells is such that the ciliated cells dominate in the ovarian end of the oviduct and the nonciliated ones in the uterine end. This is consistent with earlier investigations on the mouse oviduct (Sobotta 1895, Allen 1922, Agduhr 1927, Wen 1931, Espinasse 1935, Hartmann 1939, Fekete 1941, Henin 1941, Glass 1963, 1969, Glass and McLure 1965).

The ultrastructure of the ciliated cells conforms to that described for the mouse and similar to that in other species (Fawcett and Porter 1954, Ueki and Takahashi 1966, Nilsson and Reinius 1969). The ciliated cells had the same appearance in the different functional stages. They further lacked characteristics of secretory activity, although the great number of long microvilli can be expected to indicate good possibilities of various material exchange. The primary function of the ciliated cells seems to be to transport the ova and fluids from the ovary to the ampulla (cf.

Reinius 1969b).

Transformation of ciliated cells into nonciliated ones has been reported to take place during the estrus cycle by a loss of the cilia (Allen 1922). No stages of such a process were observed. Transformation of nonciliated cells to ciliated ones, by the development of cilia from centrioles in the apical cytoplasm or from ciliary vacuoles (cf. Brenner 1969), was not observed during the functional stages in the oviduct.

Another cell type is known as "peg-cell". The nuclei of this cell type have been described as being extruded from the epithelial membrane (Allen 1922, Espinasse 1935, in the mouse). In the present material such nuclear extrusion was not observed. The nonciliated cells in the preampulla and the ampulla, however, had a bulging apical part. The nuclei of these cells were often seen protruding above the border of the ciliated cells and surrounded by a dense cytoplasm. In the ampulla this phenomenon was pronounced, especially when its walls were not dilated by secretion. This bulging shape can be explained as an effect of less rigidity in the nonciliated cells compared to the ciliated ones, rather than as a process of nuclear extrusion. Presumably, inappropriate fixation could result in some nonciliated cells being pressed out of the epithelial lining. This could thus account for the misconception of nuclear extrusion.

Origin of tubal secretion. The oviductal epithelium and its nonciliated cells, in particular, are claimed to be responsible for the specific secretion of the oviduct. Other sources also contribute to the tubal fluid: transudation, follicular fluid, peritoneal fluid, secretions from neighbouring segments of the oviduct and epithelial cells lining the ovarian bursa (cf. Bishop 1961, 1969, Fredricsson 1969, Hamner and Fox 1969). Since the epithelial secretion probably supplies the gametes and zygotes with specific factors, the present paper will be confined to the epithelium. The term secretory cell has been avoided, since the ultrastructure of the nonciliated cells has revealed different functions of the cells in different regions of the oviduct, and not all cells show secretory activity. For further information on the ultrastructure of the epithelial cells in various mammals, see Horstmann and Stegner (1966) and Nilsson and Reinius (1969).

The interpretation of ultrastructural findings for the secretory activity of an epithelial membrane is intricate, since the secretory activity is a function of synthesis, storage and depletion. The ultrastructure may,

however, reveal the type and the amount of secretory products for example proteinaceous granules and glycogen granules in the cell; secretory activity is not demonstrated directly in this way. An epithelial origin of at least part of the oviductal secretion is suggested by the developmental changes that occurred in the granules of the ampulla.

The division of the oviduct into the four segments is made according to the ultrastructural findings and the distribution of the nonciliated cells. This would seem to be a reasonable basis for such a division, since this segmental difference in ultrastructure should imply a difference in secretory composition (cf. Nilsson and Reinius 1969). The alternative divisions of the oviduct according to the type of cells and the development of the epithelial folds and the muscular layer were regarded as less suitable. It should be noted that the nonciliated cells of the preampulla and of the ampulla are similar in appearance, but differ numerically and in the number of their secretory granules. It therefore seems adequate that the designations of these two segments only differ by a prefix. It may be noted, further, that the border between the ampulla and the isthmus is marked by the presence of a muscular sphincter forming the ampullary--isthmic junction, rather than by a distinct change in ultrastructure of the nonciliated cells.

The four segments do not quite follow the most commonly used divisions of the oviduct, viz. the infundibulum, the ampulla, the isthmus and the intramural oviduct or the so called "uterotubal junction" (Sobotta 1895, Drahn 1924, Espinasse 1935, Hartman 1939, Fekete 1941, Henin 1941, Eckstein and Zuckerman 1956, Blandau 1961, Austin 1963, Horstman and Stegner 1966, Hummel et al. 1966, Hafez and Black 1969). Thus the preampulla comprises the infundibulum and that part of the ampulla which does not distend at ovulation. The junctura consists of the proximate extramural loop of the oviduct and the intramural oviduct with the colliculus tubarius, since they are coated with a similar epithelium which differs from that of the isthmus. The junctura corresponds to the "isthmus II" of Glass (1969).

Preampulla. The nonciliated cells were few, showing a sparse number of secretory granules, which according to their ultrastructure would seem to be partly proteinaceous. No structural changes occurred during the functional stages. Therefore, the secretion by the nonciliated cells of the preampulla is probably small. This segment seems rather to

be important in the transport of the ova and fluid towards the ampulla by the cilia beating.

Ampulla. The nonciliated cells of the ampulla and the two proximate loops of the isthmus showed an extensive secretory apparatus. The fact that the number of secretory granules and dilated endoplasmic membranes had decreased in the segment containing ova and increased after they had left the ampulla, may indicate that secretory products are expelled into the lumen and that they are of significance to the ova, and may also serve as factors necessary for the fertilization and for the denudation of the ova (cf. Reinius 1969c). The granules and products of the endoplasmic cisternae may actually correspond to the ampullary factor necessary for the development of the ovum such a factor does not exist in the isthmus and is most abundant during early metestrus (Whittingham 1968a,b).

The granules are likely to be of a proteinaceous nature as evidenced by the ultrastructure, and they might in addition contain mucoproteins and/or mucopolysaccarides, since they are PAS-positive and diastase resistent (Fredricsson 1969).

The presence of ova seems to evoke a specific environment in the ampulla, since the ampullary fluid then distends the walls. Further, the ampullary-isthmic junction can be expected to be closed (cf. Reinius 1969b).

Isthmus. The nonciliated cells in the main part of the isthmus lacked the pronounced protein secretory apparatus of the ampullary cells, but contained glycogen granules. The close relation of these glycogen granules to the agranular endoplasmic reticulum is indicative of a function of the latter in the glycogen synthesis and degradation (cf. Fawcett 1966). The quantitative changes of the glycogen granules during the functional stages can be interpreted as evidence of the secretion of carbohydrates into the lumen for utilization by the spermatozoa and the zygotes. In fact, there is an increase of glycogen in the 2-cell zygotes when they are lying in the isthmus (Stern and Biggers 1968). Thus, the source of the glycogen of the zygotes may be the carbohydrate rich secretion in the isthmus (cf. Reinius 1969c). The mechanism of this secretion is not known.

The lumen is distended by secretion in those loops where the zygotes are present, but otherwise it is closed. This suggests that the isthmus secretion provides the zygotes in the isthmus with a specific environment. Also, secretions from the ampulla certainly are transported together with the ova into the isthmus.

Junctura. The nonciliated cells of the junctura showed regular and tight microvilli, invaginations of the apical membrane, coated vesicles, apical vesicles, lysosomal structures and a large Golgi complex - all structures which in combination can be regarded as features of absorbing cells (cf. the cells in the ductus epididymidis (Nicander 1965, and Friend and Farquhar 1967). The number of these structures in the cell depended upon the stage. At the time of sperm cell migration and the passage of the zygotes, when much fluid lay in the uterus and at the time when the secretions in the isthmus disappeared, the signs of absorption were more pronounced.

The narrow or closed lumen indicates that there is little or no fluid in the junctural segment. A closed lumen of this segment has also been indicated by a previous experimental study (Alden 1943).

An absorptive activity of the cells in the junctura is indicated by their structure, the changes during the functional stages, the closed lumen and the finding that the zygotes at the stage when they are passing through this segment do not need any exogenous protein (Brinster 1969). The passage probably lasts for several minutes to a few hours (Reinius 1969b). An absorptive function of the oviductal epithelium in the ampulla and/or isthmus of the rat and man has been reported earlier (cf. Vokaer and Vanderbeken 1960, Wolf 1960, Fredricsson and Björkman 1962).

The role of the junctura seems to be one of a barrier between the isthmus and the uterus. This is clear from the finding of the almost closed lumen and from the absorptive activity of the nonciliated cells. The purpose of this segment may, further, be one of limiting and selcting a few actively migrating spermatozoa during their passage into the oviduct (Reinius 1969a).

The segmental differences in the character of the oviductal secretion hence indicated that ova/zygotes are surrounded by a specific tubal secretion fitted for each stage of development. If the transport is disturbed, e.g. by exogenous hormones, the optimal conditions for the development of the ova/zygotes may thus be impaired.

No local structural differences within a segment that might have been produced by the presence of the ova/zygotes were noted (cf. Fredricsson 1969).

Collected tubal secretion from the whole oviduct seems to be a mixture

of too many different components to be useful for the study of the optimal environment of zygotes and spermatozoa. Such studies rather have to be performed with samples from the different segments and at the functional stages when the gametes or ova/zygotes lie within the segments.

SUMMARY

The technique of perfusion fixation and plastic embedding was applied to the oviduct at the functional stages when the gametes and zygotes lay in situ in the oviduct, and appeared to yield relevant morphological information both with light and electron microscopy.

The ultrastructure of the nonciliated cells varies in the different segments of the oviduct and changes during the various functional stages. The nonciliated cells seem to contribute to a part of the oviductal secretion and hence have significance for the ova/zygotes.

Four segments are distinguished on the basis of the ultrastructure and distribution of the nonciliated cells.

The preampulla has mainly ciliated cells and a few nonciliated cells that have few or no signs of secretory activity.

The ampulla contains many nonciliated cells that show proteinaceous secretion, which varies during the functional stages.

The isthmus has mainly nonciliated cells which are of two types. One is similar to those of the ampulla and is located in the loops next to the ampulla; the other, which occupies the main part of the isthmus, probably elaborates a carbohydrate rich secretion which varies during the functional stages.

The junctura, consisting of the intramural oviduct and the proximal extramural loop, has only nonciliated cells which probably have an absorptive rather than a secretory activity. The lumen of the junctura is almost closed. This segment should act as a barrier between the oviduct and the uterus.

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LEGENDS

Fig. 3. Preampulla when ova lie in the ampulla, Day 1/10hr. The epithelium consists of mainly ciliated cells, but a few nonciliated cells (Nc) are seen. Arrows point to large lipid granules or vacuoles of ciliated cells. Light microscopy x 900.

Fig. 4. Preampulla, Day 1/16hr. Apical part of a ciliated cell with motile cilia (Mc) and long microvilli (Mv). Endoplasmic reticulum (Er); ribosomes (R). Electron microscopy x 50,000.

Fig. 5. Ampulla with two pronuclear ova, Day 1/15hr. The epithelium to the right consists of mainly nonciliated cells (Nc), but some ciliated cells (Cc) are seen. The ova, surrounded by degenerating follicle cells, lie in a wide lumen that is filled with fluid. Light microscopy x 400.

Fig. 6. Ampulla when pronuclear ova lie in the segment, Day 1/16hr. Apical part of a nonciliated cell containing granules (Gr), probably proteinaceous, and dilated cisternae of the endoplasmic reticulum (Er) containing a less electron dense substance. Microvilli (Mv); mitochondria (M). Electron microscopy x 20,000.

Fig. 7. Ampulla when 2-cell zygotes lie in the isthmus, Day 2/15hr. Nonciliated cells are seen to the left and one ciliated cell to the right with motile cilia (Mc). The nonciliated cells contain many granules (Gr), probably proteinaceous, and many dilated cisternae of the endoplasmic reticulum (Er) containing a less electron dense material. Mitochondria (M). Electron microscopy x 7,000.

Fig. 8. Isthmus with three 2-cell zygotes, Day 2/16hr. The epithelium forms circular folds. The lumen is relatively wide and contains fluid. Light microscopy x 400.

Fig. 9. Isthmus with part of a 2-cell zygote, Day 2/16hr. The epithelium consists mainly of nonciliated cells. A few ciliated cells may occur in the grooves of the epithelial folds. Connective tissue stains dark. Light microscopy x 900.

Fig. 10. Isthmus when 2-cell zygotes lie in the segment, Day 2/16hr. Nonciliated cell containing at this stage many glycogen granules (Gg). Nucleus (N); microvilli (Mv); mitochondrion (M); Golgi complex (G); granular endoplasmic reticulum (Er); agranular endoplasmic reticulum (Aer); ribosomes (R); multivesicular body (Mb). Electron microscopy x 30,000.

Fig. 11. Junctura and upper part of the uterus, when 5- to 8-cell zygotes lie in the segment, Day 3/17hr. Note the narrow lumen of the junctura, both the intramural (Ji) and the extramural part (Je) and the narrow uterine opening of the oviduct (arrow) at the colliculus tubarius (Ct). Lumen (L) of the uterus. Fixation was performed by the perfusion,technique causing no shrinkage of the tissue and leaving the vessels (Ev) without any blood corpuscles. Light microscopy x 30.

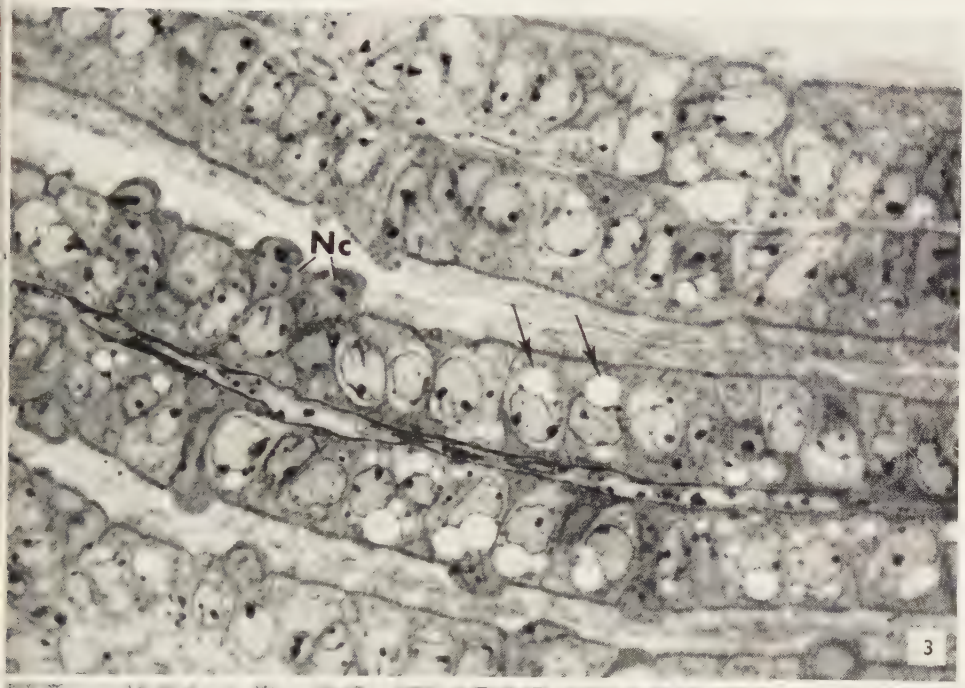
Fig. 12. Junctura and upper part of the uterus at the time of zygote passage, Day 3/17hr. Fixation was performed by pouring the fixative into the abdomen, giving shrinkage of the tissues and wide lumina in the uterus (L) and the intramural part of the junctura (Ji). Arrow indicates the uterine opening of the oviduct. Extramural part of the junctura (Je); proximal part of the isthmus (I). Light microscopy x 30.

Fig. 13. Junctura, intramural part, with an 8-cell zygote, Day 3/17hr. The epithelium consists of only nonciliated cells and lies closely to the zygote. Light microscopy x 400.

Fig. 14. Junctura, when 2-cell zygotes lie in the isthmus, Day 2/16hr. Apical part of a nonciliated cell. Microvilli (Mv) and apical vesicles (Ve) are few compared with the stage when the zygotes lie in the junctura (cf. Fig.14). Mitochondria (M); junctional complex (Jc); granulated endoplasmic reticulum (Er). Electron microscopy x 15,000.

Fig. 15. Junctura, the day of fertilization when ova lie in the ampulla, Day 1/16hr. Basal part of nonciliated cell containing many glycogen granules (Gg) and lipid granules (Lg). Basal membrane (Bm). Electron microscopy x 13,000.

Fig. 16. Junctura with a 6-cell zygote, Day 3/16hr. The microvilli (Mv) of the nonciliated cells lie in contact with the zona pellucida (Zp) of the zygote. The apical part of the cell is filled with many vesicles (Ve); coated vesicles (Cv); multivesicular bodies (Mb) and dense bodies (Db). Mitochondria (M); Golgi complex (G); granular endoplasmic reticulum (Er); microtubulus (Mt); junctional complex (Jc). Electron microscopy x 18,000.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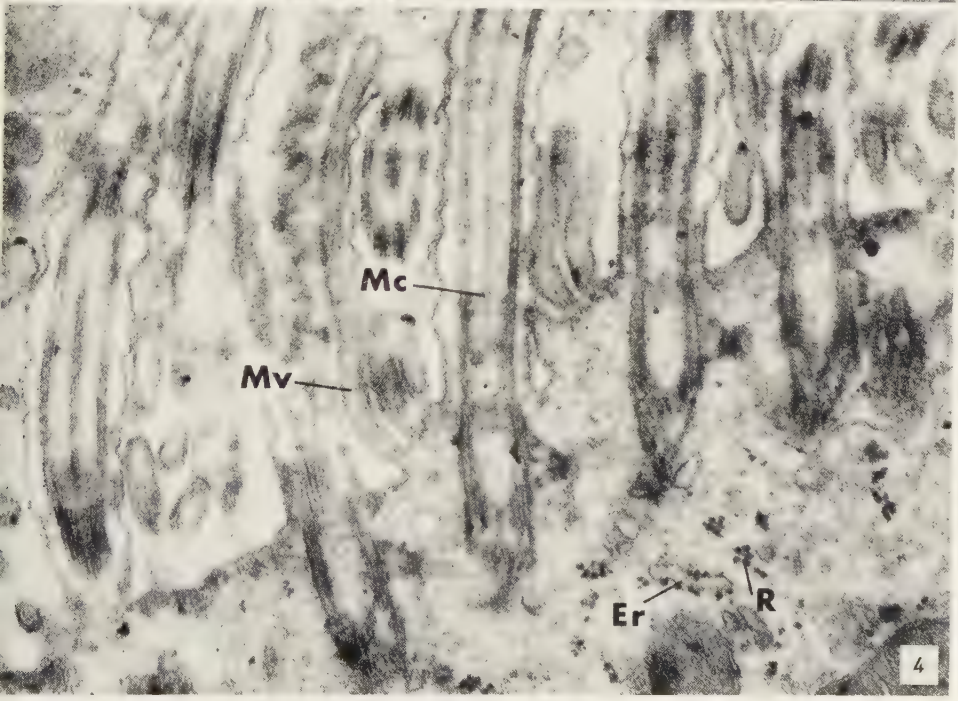
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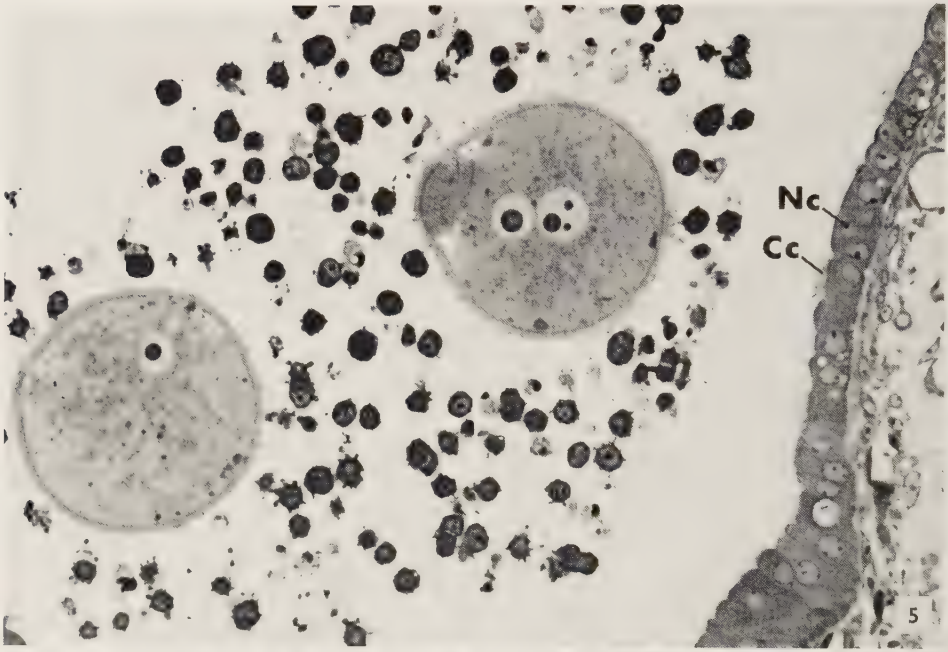
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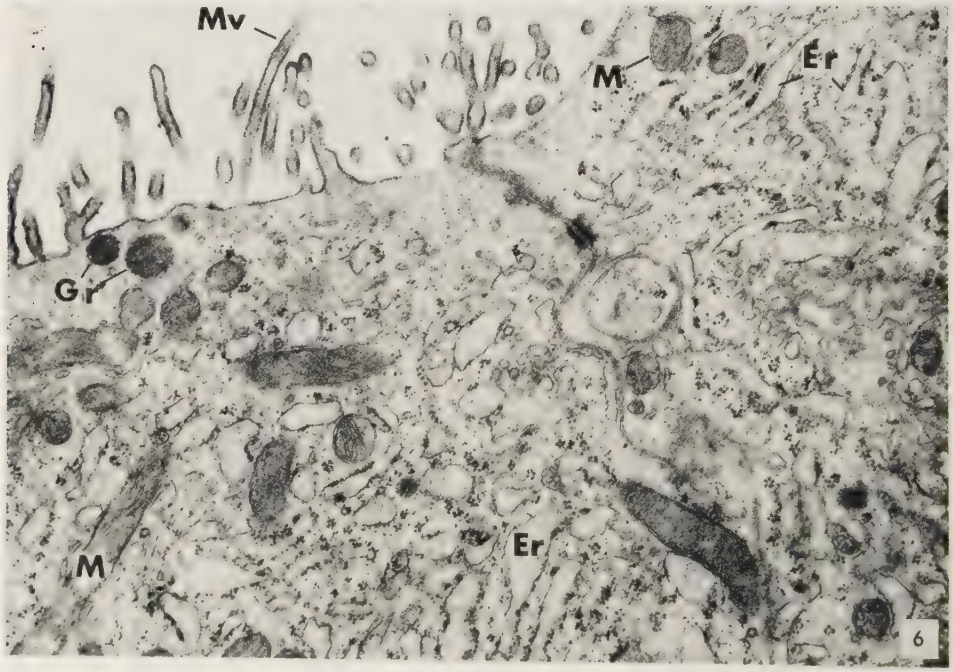
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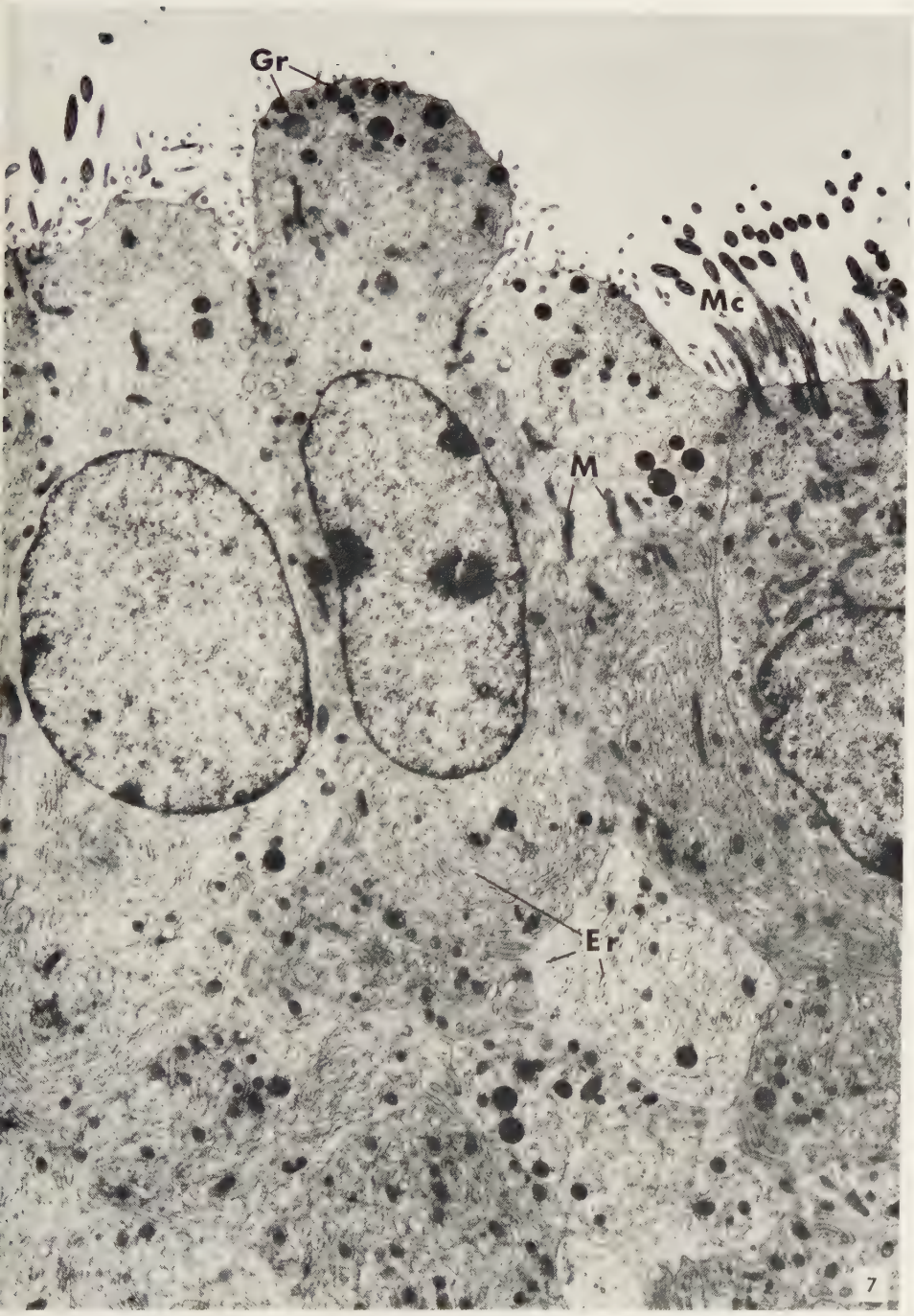
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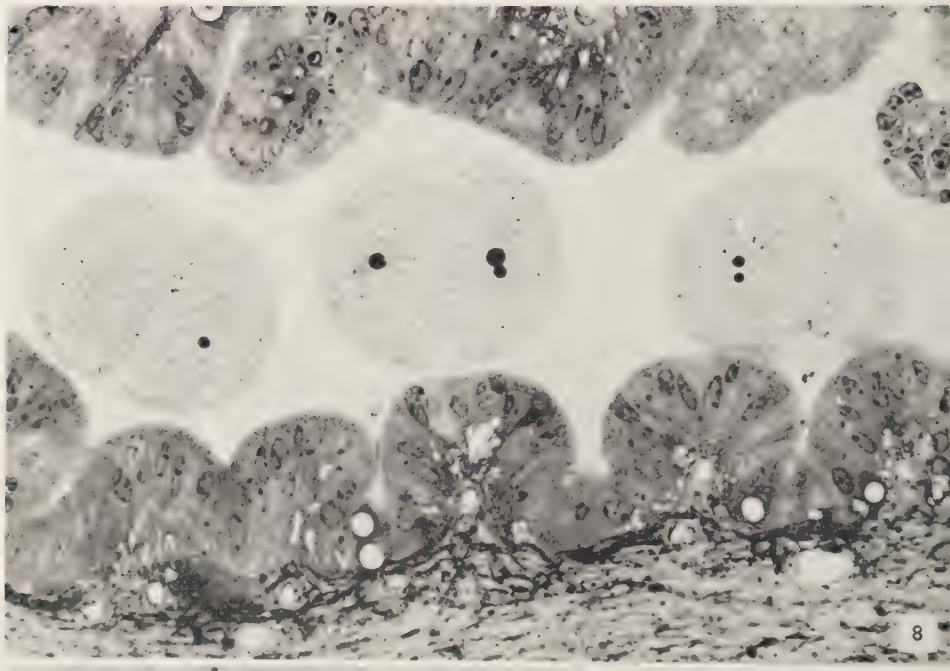
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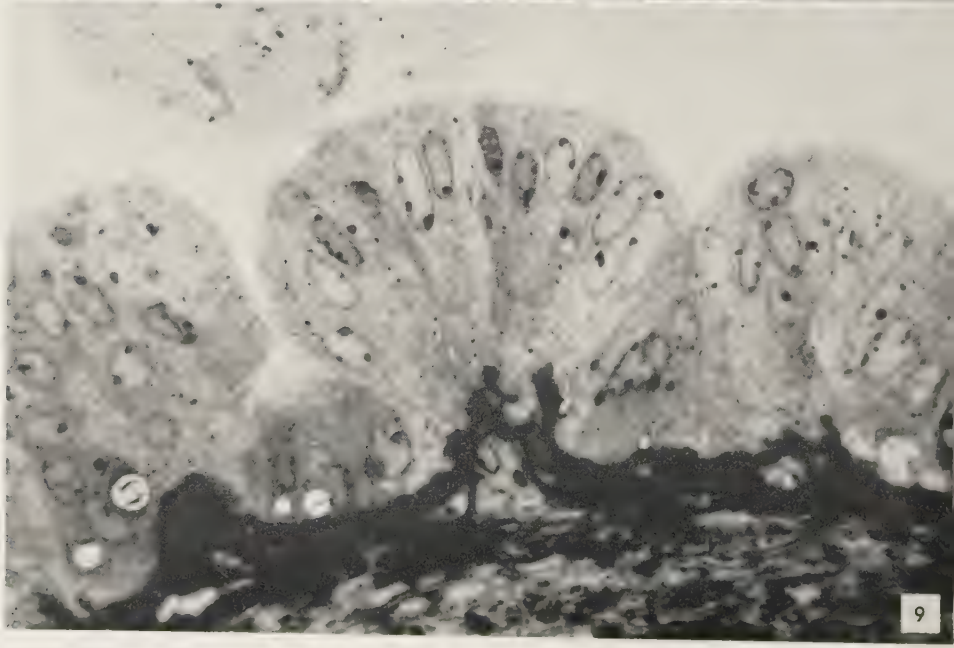
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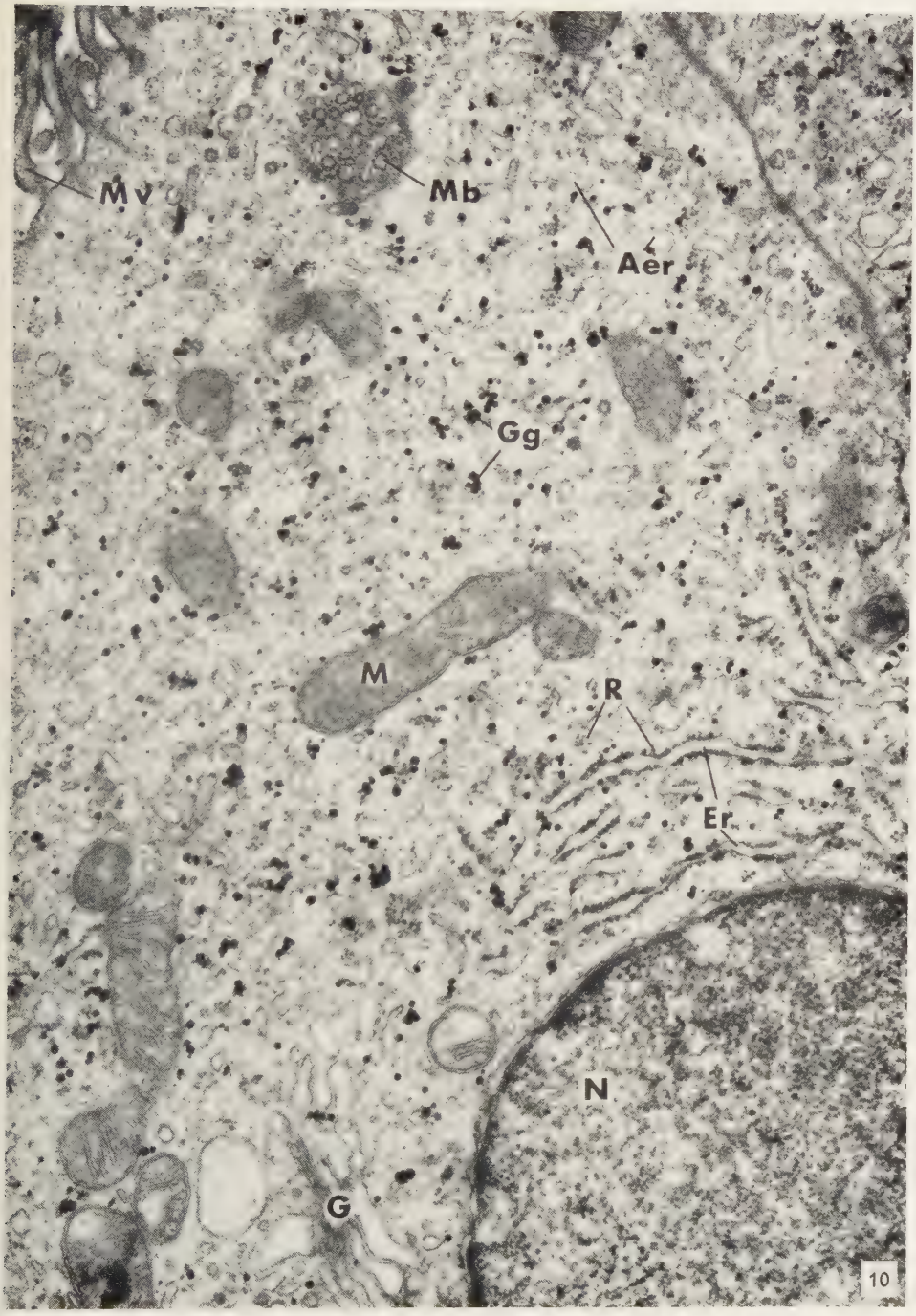
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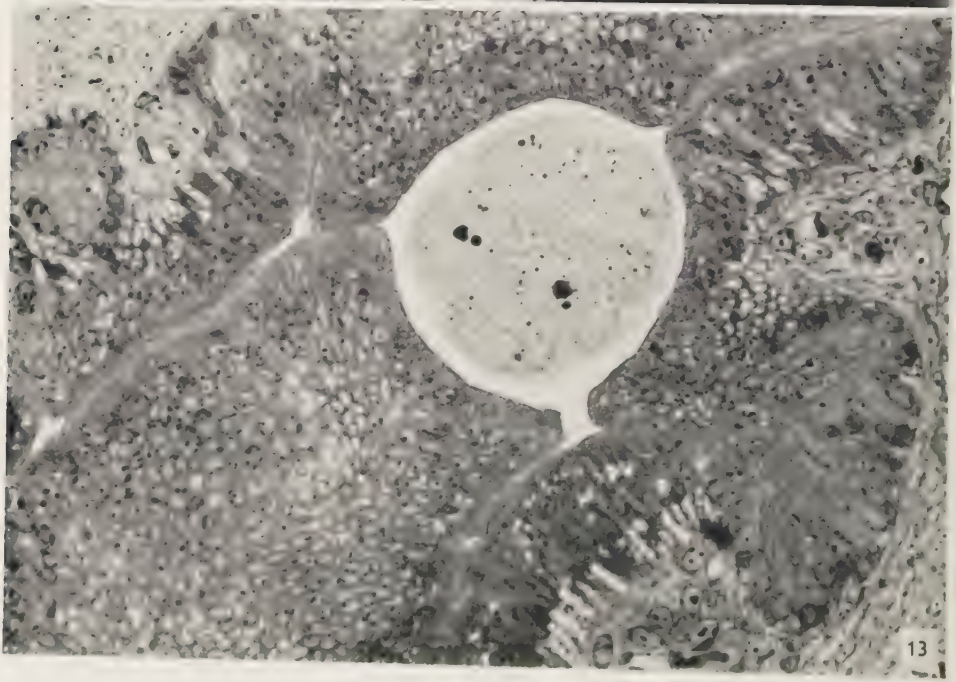
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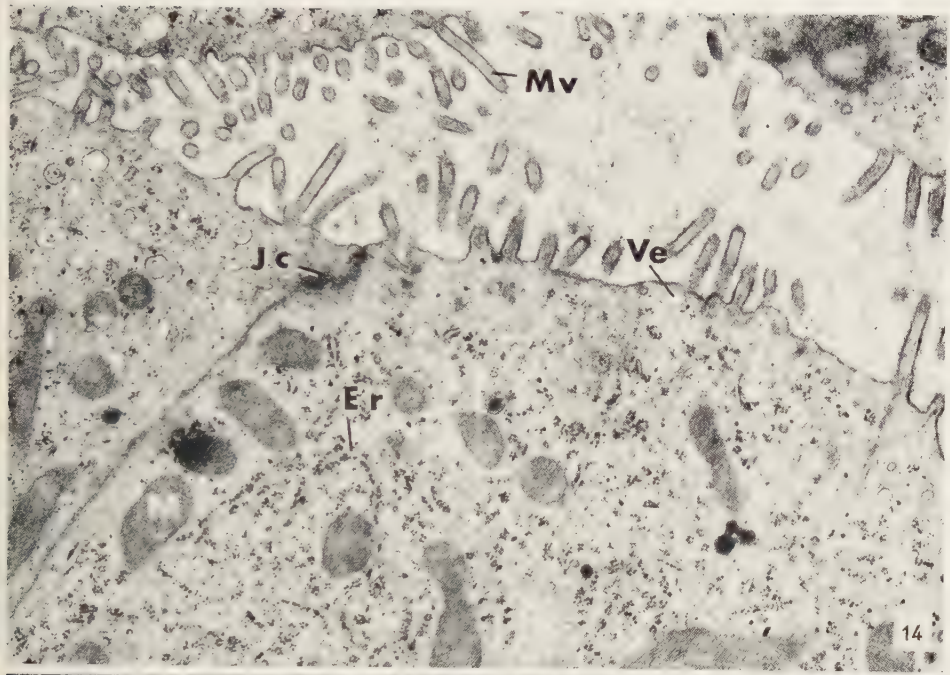
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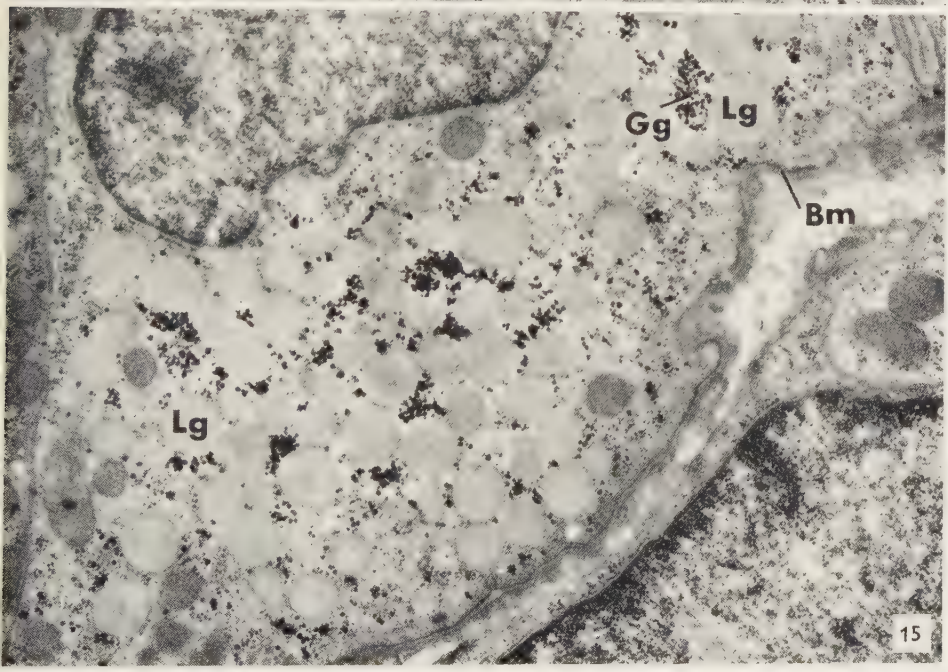
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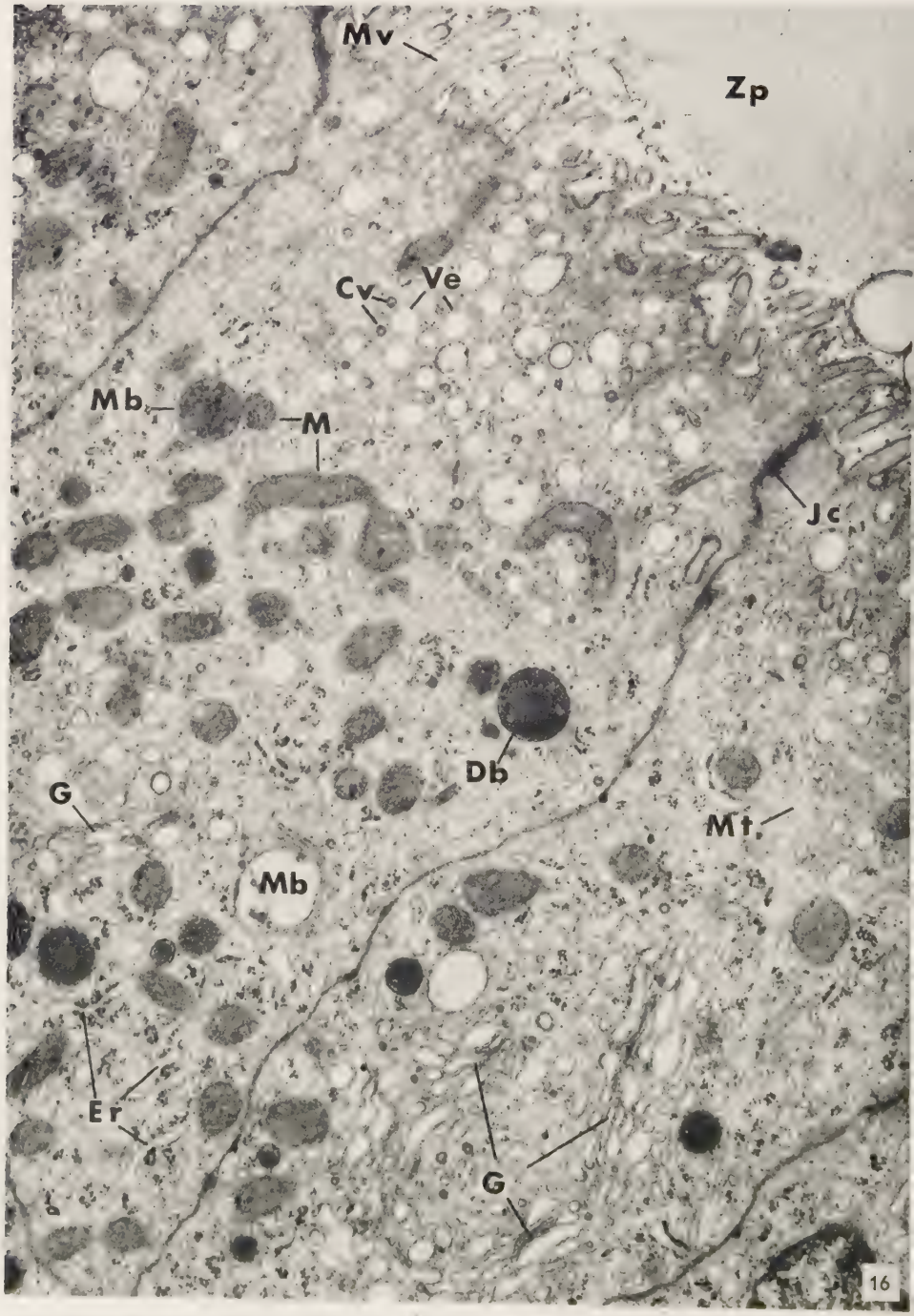
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16

II. SPERM CELL MIGRATION

Sperm cell migration in the female genitale tract of the mouse and the rat gains special interest at the uterotubal junction, since both morphological studies (Tafani 1889, Sobotta 1895, Sobotta and Burchard 1910) and counts of the actual number of spermatozoa have revealed a large number of spermatozoa in the uterus but a small number in the oviduct (Lewis and Wright 1935, Blandau and Money 1944, Austin 1948, Blandau and Odor 1949, Braden and Austin 1954a,b, Braden 1958). Also in other mammals the number of spermatozoa in the oviduct is small (for references see Bishop 1961 and Blandau 1969). The mechanisms that make the spermatozoa enter are unsolved. It is thus not known whether sperm cell motility itself plays any role or whether there are physical or chemical factors that determine the transport (Blandau 1969).

The decrease in number of spermatozoa at the uterotubal junction in the mouse and rat is chiefly attributable to a diminution of the size of the lumen and to the special structural formation at the uterotubal junction, viz. the colliculus tubarius, (Powierza 1912, Fischel 1914, Drahn 1924, Andersen 1928, Alden 1943, Sobrero 1963, Kuhlmann 1964, Hummel et al. 1966, Hook and Hafez 1968, Hafez and Black 1969). Conventional histological techniques have always shown the uterine opening of the oviduct on the colliculus and the lumen of the intramural oviduct to be relatively wide (Sobotta 1895, Powierza 1912, Fischel 1914, Allen 1922, Agduhr 1927, Andersen 1928, Lee 1928, Hummel et al. 1966, Hook and Hafez 1968, Hafez and Black 1969, and others). This wide opening and lumen are not correspondent with the limiting effect of the spermatozoon entrance at the uterotubal junction.

However, the development of new preparation techniques with rapid fixation by perfusion, embedding of large specimens in plastic and methods for their sectioning have made it possible both to preserve the structural relationships in the oviduct nearly unchanged, and to study the oviduct and the uterus of the mouse with gametes and zygotes *in situ* (Reinius 1967, 1969a,b).

The aim of this report is to describe the relationship of the spermatozoa and the epithelium of the uterus and the oviduct with special consideration of the junctura, the intramural oviduct and the proximate extramural loop of the oviduct. From the results new mechanisms will be suggested, both for the entrance of the sperm cell into the oviduct and for the sperm cell migration through the oviduct.

MATERIAL AND METHODS

The material constitutes a part of the series of animals used for studies of the oviductal epithelium and oviductal ova and zygotes (Reinius 1969a,b). Males and virgin females of the CBA mouse strain were used and conditioned to artificial light from 05 to 19 hr. Pregnant females were selected after observed copulation (two animals) or after the finding of a vaginal plug (seven animals). Day 1 was the day when the vaginal plug was found. The animals were fixed from 45 min to about 5 hr after copulation (see Table 1).

Fixation was performed by aortic perfusion with 2.5% glutaraldehyde in Sørensen phosphate buffer, pH 7.3, osmolality about 450 mOsm.

The genital organs were dissected out and from each animal one oviduct was divided into four segments: the junctura with the attached upper part of the uterine horn, the isthmus, the ampulla, and the preampulla. The other oviduct with the ovary and upper part of the uterine horn were kept intact in order to control possible traumatic effects of the fine dissection. Postfixation was performed in 1% osmium tetroxide in the Sørensen buffer, dehydration in ethanol, and embedding in Epon.

Sectioning for light microscopy was performed on a Porter-Blum ultramicrotome. For ordinary electron microscopy large areas, covering about half the cross section of the oviduct, were sectioned on an LKB Ultrotome. For further details of the technique, see Reinius (1969a).

Scanning electron microscopy was used to study the unsectioned colliculus tubarius. Fourteen animals were fixed by perfusion at Day 1/10hr. The colliculus tubarius was exposed from the uterine end by removing a coagulated mass of semen and uterine secretion and by careful dissection under a stereomicroscope in glutaraldehyde. Dehydration after postfixation was performed by freeze-drying. The specimens were then glued to special holders, coated with gold in vacuum, and studied in a Cambridge Instrument Company, "Stereoscan" scanning electron microscope using a beam accelerating voltage of 5 kV (cf. Barber and Boyde 1968).

Table I. Data on animals fixed for study of sperm cell migration

Animal No.	Time of Pairing	Time of Fixation Day/Hr	Hrs after Copulation	Stage of Development	Location of Ova
1	21.00-21.15*	0/22	3/4	I Spindle	Ovary
2	21.00-22.30*	1/00	2 1/2	II "	"
3	21 - 23hr	"	1 - 3	I "	"
4	"	"	1 - 3	I "	"
5	22 - 01hr	1/01	0 - 3	I "	"
6	23 - 04hr	1/05	5**	II "	Ampulla
7	"	"	5**	II	"
8	"	"	5**	II	"
9	"	"	5**	II	"

* The later time refers to the time of observed copulation
** Time estimated

RESULTS

The spermatozoon head of the mouse is flat and has the hook--shaped anterior end typical of the Rodentia (Retzius 1909, Austin and Bishop 1958). The head was found to be about 7 to 8μ long, 3μ broad and 0.7 to 0.8μ thick. The mid-piece was about 20μ long and 1μ thick, and this and the rest of the tail possessed the basic pattern of longitudinal filaments of a flagellum with the 2 x 9 + 2 array. The mitochondria of the mid-piece had internal cristae and were filled with electron dense material (Fig.8).

The uterus generally had a wide lumen, distended by fluid and spermatozoa at the time of ovulation and mating. The colliculus tubarius then protruded into the uterine lumen (Figs.3 and 9). In two animals that had not ovulated, the uterine cavity was narrow and had little secretion (Fig.2), but spermatozoa were nevertheless found in the upper part of the uterus and in the junctura. The distribution of spermatozoa was even in the wide uterine lumen (Fig.4). However, the spermatozoa were concentrated in clefts and crypts of the uterine wall, with their heads closely packed together and their tails merging into the uterine lumen (Figs.4,5 and 10).

The oviduct of the mouse consists of four segments: the junctura, the isthmus, the ampulla and the preampulla, differing in their fine structure and in the distribution of the nonciliated cells (Reinius 1969a). The junctura comprises the intramural part with the uterine opening of the oviduct at the colliculus tubarius, and the proximate extramural loop of

the oviduct (cf.Figs.2 and 3). The junctura has only nonciliated cells (cf.Figs.6, 7 and 8).

The uterine oviduct opening formed a narrow, often semilunar slit (Figs.5, 9 and 11). The opposing apical parts of the epithelial cells at the opening lay close together, but around the opening the cells were hypertrophied and formed small villi at the time of ovulation. This made the oviduct slit a little wider in the outer part, and the slit became funnel-shaped in a transverse section. Spermatozoa were concentrated in the uterine opening of the oviduct in the same way as in the clefts and crypts of the uterus.

The junctura lumen was star-shaped and narrow, and the microvilli (0.5 to 1.0μ long) of the opposing epithelial cells usually met (Figs.5, 6 and 7). In the central part of the lumen wider spaces of up to 2 to 3μ were formed between the tops of the microvilli (Figs.6 and 8). Many spermatozoa lay at the uterine end of the junctura and almost formed a file (Fig.5); but few are seen at the ovarian end of the junctura. The relationship between the spermatozoa and the epithelium could be seen in the electron microscope: the sperm tails, main- and mid-pieces, and also heads were found to be in direct contact with the microvilli of the opposing epithelial cells, or in the somewhat wider luminal spaces (Figs. 7 and 8).

The isthmus lumen was wide in some places and narrow in others, few spermatozoa were seen and these were mainly located in the central luminal space. No spermatozoa were seen in the lumina of the ampulla and the preampulla, outside the follicle cell mass.

Within the epithelial cells or below the uterine or oviductal epithelium no spermatozoa were observed.

DISCUSSION

The spermatozoa observed in this study should be normal and viable at the time of fixation, since spermatozoa enter the oviduct more than one hour after copulation and retain their fertilization capacity up to 12 hr after copulation in the female genital tract of the mouse and rat (Leonard 1950, McGaghey et al. 1968). The passage of the spermatozoa in the oviduct may be influenced by muscular contractions of the oviductal wall (Bishop 1961, Noyes 1965, Blandau 1969), but the motility rate of the spermatozoa is adequate for the time of passage in the mouse ovi-

duct (Agduhr 1927, Milovanov 1934, cit. Bishop and Walton 1960, Lewis and Wright 1935, Braden and Austin 1954b). It is relevant, therefore, to discuss those structural conditions of the oviduct that might influence an active entrance and migration of the spermatozoa.

The technique of perfusion and plastic embedding has proved satisfactory for preservation of a variety of tissues in electron microscopy (for ref. see Fahimi 1967). This and similar techniques have also been shown to maintain to a high degree the in vivo spatial relations of the tissues (Maunsbach 1966, Forssmann et al. 1967, Reinius 1969a,b). Thus, the width of the uterine opening of the oviduct and the lumen of the oviduct should be reliable.

Using this improved morphological method, the present study has shown firstly, that the uterine opening of the oviduct and the lumen of the junctura are almost closed and much narrower than has been previously demonstrated (Figs.2, 3, 5, 6, 7, 8, 9, and 11)(Sobotta 1895, Powierza 1912, Fischel 1914, Allen 1922, Drahn 1924, Agduhr 1927, Andersen 1928, Lee 1928, Hummel et al. 1966, Hook and Hafez 1968, Hafez and Black 1969, and others). This holds both during sperm cell migration and passage of the zygotes (Reinius 1969a,b). Secondly this study has shown, that there is a concentration of spermatozoa in uterine clefts and crypts, in the narrow oviduct opening, and at the uterine end of the junctura(Figs. 1, 4, 5, 10 and 11). These observations suggest new mechanisms both for the entrance of sperm cells into the oviduct, and for the sperm cell migration through narrow passages, as in the junctura.

The mechanism of sperm cell entrance into the oviduct is based on the assumption that the spermatozoa actively migrate in straight lines at random when lying freely in fluid; when they hit a wall they either change direction or follow the surface; and when they reach a slit-like structure or a crypt, they are directed by the walls and by their own motility further into these structures. These properties of the spermatozoa have been indicated by Noyes (1965) and by studies on the migration of bull spermatozoa (Gray 1958, Rikmenspoel 1962) and the entrance of spermatozoa into the micropyle of certain insects and fishes (Yanagimachi 1957, Ginsburg 1963) or into the specific "tuft" of the lamprey egg (Kille 1960).

The entrance of sperm cells probably is due to this mechanism, since many spermatozoa are concentrated in uterine clefts and crypts, and in

the uterine oviduct opening. Moreover, the wider outer part of the slit-like oviduct opening may even increase the chance of hitting it.

The significance of the narrow slit-like oviduct opening is that it facilitates the entrance of a limited but sufficient number of normally migrating spermatozoa and that it probably eliminates pathological spermatozoa. Thus motility alone should be sufficient for the entrance of the spermatozoa into the oviduct. This is accordant with other studies (Leonard and Perlman 1949, Braden and Glueckson-Welsch 1958, Braden 1960, Blandau and Rumery 1964), although it has also been reported that a small number of immotile and heterologous spermatozoa and inert substances can enter (Leonard and Perlman 1949, Howe and Black 1963, Marcus 1965).

Other mechanisms for the entrance of spermatozoa into the oviduct have been suggested. The colliculus tubarius has been described as acting as a valve which should open from time to time by the relaxation of a sphincter; spermatozoa should then be forced into the oviduct opening by muscular activity of the uterus (Kuhlmann 1964). However, no sign of change in the dimension of the lumen has been observed even at other gestational stages except locally where the zygote passes (Reinius 1969a, b). Retrograde injection of fluid into the uterus through the junctura indicates a closed lumen, but spermatozoa will nevertheless enter the oviduct at any stage in the cycle (Noyes 1965, Blandau 1969). However, the retrograde injection is disadvantageous in studying the uterine opening of the oviduct, since unphysiological intrauterine pressures are created. Indeed, the form of the colliculus tubarius and the narrow lumen of the junctura should work as a valve on retrograde injection and give false information (cf. Hafez and Black 1969). Also positive rheotaxis (Yamane and Ito 1932, Walton 1952, Bishop and Austin 1957, Bishop and Walton 1960) and positive chemotaxis have been mentioned. The latter, however, has not been shown to occur in mammals (Bishop and Austin 1957). The fact that spermatozoa are similarly concentrated in uterine clefts and crypts and in the slit-like uterine opening of the oviduct speaks against these theories.

The mechanism of sperm cell migration through narrow passages is based on the assumption that the spermatozoa, when arriving at narrow slits, use the walls as supports for the sperm tail movement and thereby become directed by the surfaces. These properties of sperm movement

have been indicated by sperm cell migration through the narrow channels of the cervical mucus (Tampion and Gibbons 1962, Odeblad 1968) and the passage between the follicle cells of the newly discharged ovum. These radially arranged follicular cells around the ovum tend to direct the movements of the approaching spermatozoa towards the egg (Austin and Braden 1952, Blandau 1961, Braden 1962).

The sperm cell migration through the junctura might work according to this mechanism, since the junctura has a narrow lumen with closely apposed epithelial walls in close contact with sperm cells (Figs.5, 6, 7 and 8). The spermatozoa probably are directed to the ovarian end of the oviduct by the longitudinal arrangement of the epithelial folds.

A narrow lumen of the junctura may be a rather common phenomenon, since it is seen also in the rat and the guinea-pig after using the perfusion and plastic embedding technique. In the bat, Glossophaga soricina, and man the intramural region of the oviduct has been found to be similarly narrow after fixation by immersion (Nilsson and Reinius 1969, and unpublished). Further, closed lumina of the junctura and the isthmus in the rabbit have been indicated by experimental studies (Hafez 1963, Brundin 1965). Thus, a similar mechanism of sperm cell migration may work generally at the uterine end of the oviduct in mammals.

The significance of the junctura may not only be to facilitate spermatozoon passage, but also to separate two environments, that of the uterus and that of the isthmus (Reinius 1969a).

In the further passage of the spermatozoa in the isthmus one has to consider, besides the small number of spermatozoa and wide and narrow lumina, circular folds with a few ciliated cells located basally and a thick muscular coat (Reinius 1969a). The furrows created by the epithelial folds should be traps for the spermatozoa, but spermatozoa were rarely found in the furrows, probably prevented from being trapped by the basally located ciliated cells. The transport may be facilitated by muscular activity of the oviductal wall (cf. Bishop 1961, Noyes 1965, Blandau 1969), although the motility of the spermatozoon is adequate for the time of passage (see discussion above). In animals with large oviducts, however, such as the sheep and the cow, muscular movements of the oviduct are mainly responsible for the propagation of the spermatozoa (for ref. see Bishop 1961). The passage through the ampullary-isthmic junction may be mainly an effect of the spermatozoon itself, since this

junction is of similar narrowness as the junctura (Reinius 1969a).

SUMMARY

The mouse oviduct and uterus with spermatozoa in situ were examined by light and electron microscopy after fixation by aortic perfusion with glutaraldehyde, postfixation in osmium tetroxide, and embedding in Epon.

The uterine opening of the oviduct is formed as a narrow semilunar slit. The junctura, that is the intramural oviduct and the proximate extramural loop, has a narrow lumen, where the microvilli of the opposing epithelial cells meet or lie a few microns apart. Spermatozoa are concentrated in clefts and crypts of the uterus, in the oviduct opening, and in the uterine end of the junctura. Few spermatozoa are observed in the ovarian end of the oviduct.

New mechanisms are suggested from the results both for the entrance of sperm cells into the oviduct and for sperm cell migration in the oviduct. The narrow oviduct opening makes possible the entrance of a limited but sufficient number of normally migrating spermatozoa and probably eliminates pathological spermatozoa. In the junctura the narrow lumen and the longitudinal arrangement of the epithelial folds facilitate the passage of actively migrating spermatozoa.

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Fig. 1. Schematic drawing of the upper part of the uterus, the uterine lumen (UL), the colliculus tubarius (CT) with the opening of the oviduct (arrow) and the junctura (J). A representative distribution of the spermatozoa is shown, with higher concentrations in the uterine clefts and crypts, in the narrow opening of the oviduct, and in the uterine end of the junctura.

LEGENDS

Fig. 2. Upper part of the uterine horn with junctura, Day 1/00hr. The uterine lumen (L) is narrow and has little secretion. Spermatozoa have already reached the oviduct. The arrow indicates the uterine opening of the oviduct. The junctura is marked with straight lines. Light microscopy x 28.

Fig. 3. Upper part of the uterine horn with junctura, Day 1/01hr. The uterine lumen (L) is distended by fluid and spermatozoa. The arrow indicates the uterine opening of the oviduct at the colliculus tubarius (Ct). Light microscopy x 28.

Fig. 4. Uterine wall at the distended stage, Day 1/01 hr. The epithelium forms small clefts, where spermatozoa are concentrated (arrows). In the uterine lumen above spermatozoa are fairly evenly distributed. Light microscopy x 390.

Fig. 5. Part of colliculus tubarius sectioned longitudinally, Day 1/01hr. The opening of the oviduct (➤) and the narrow lumen of the junctura are seen, where heads of the spermatozoa are distinguished as black dots. A cleft on the colliculus is filled with trapped spermatozoa (➧). The uterine lumen to the right. Connective tissue and smooth muscle cells are dark stained. Light microscopy x 390.

Fig. 6. Junctura, intramural part cross-sectioned, Day 1/05hr. The epithelium consists only of nonciliated cells. The lumen is star-shaped and narrow and the surfaces of the epithelium usually meet. In the central part of the lumen spaces are formed not wider than a few μ (arrows). Light microscopy x 400.

Fig. 7. A spermatozoon head (Sh) in the narrow lumen of the junctura, Day 0/22 hr. Microvilli of the epithelial cells lie in direct contact with the spermatozoon. Electron microscopy x 12,500.

Fig. 8. A spermatozoon head (in the middle), a mid-piece (to the left) and a main-piece (to the right) in the central part of the junctura, Day 1/05hr. The lumen is up to 2 to 3μ between the microvilli (Mv). Electron microscopy x 20,000.

Fig. 9. Upper part of the uterine horn with the colliculus as seen in the scanning electron microscope, Day 1/10hr. The V-shaped uterine opening of the oviduct forms a wider excavation than the many clefts and crypts on the colliculus and in the uterine epithelium. x 50.

Fig.10. Tails of spermatozoa merging out of a cleft on the colliculus, Day 1/10hr. The microvilli of the nonciliated cells are visible. Scanning electron microscopy x 2,000.

Fig.11. The semilunar slit of the uterine oviduct opening, Day 1/10hr. The filamentous structures that fill the excavation of the opening and to a lesser extent the small clefts on the colliculus are mainly the tails of spermatozoa (S), that have been trapped in the excavations. Each small area (arrow) is the surface area of one cell. Scanning electron microscopy x 275.

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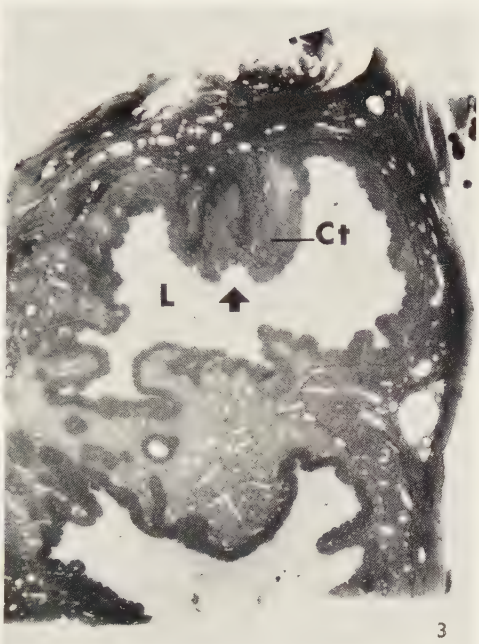

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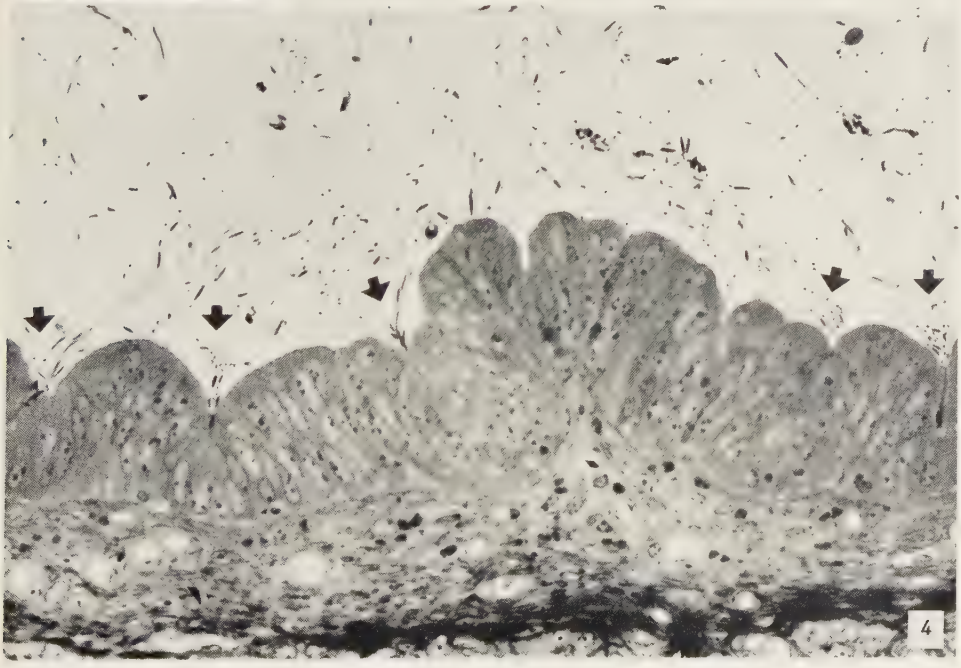

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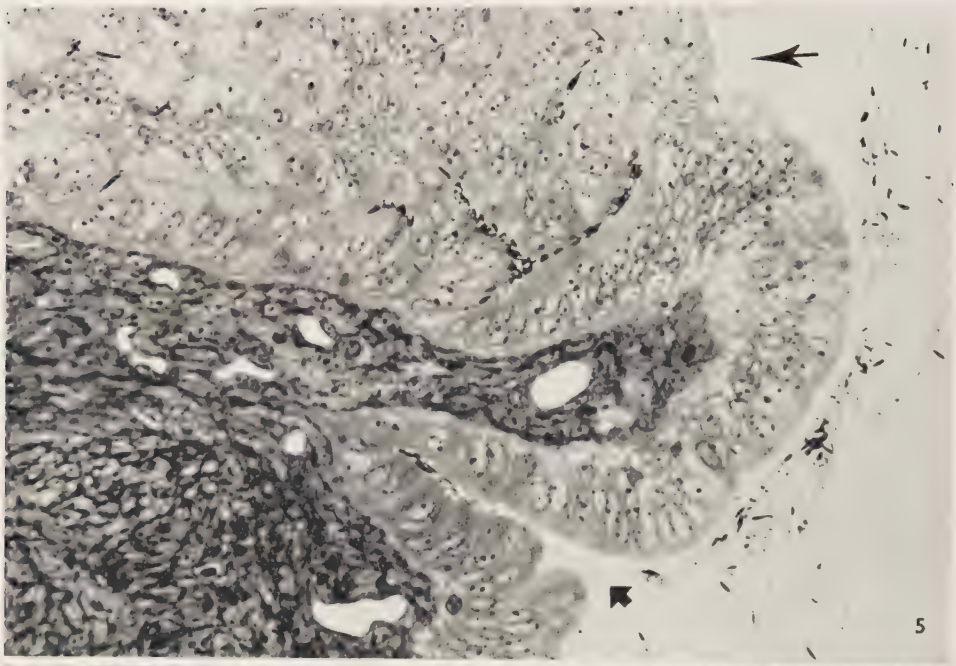

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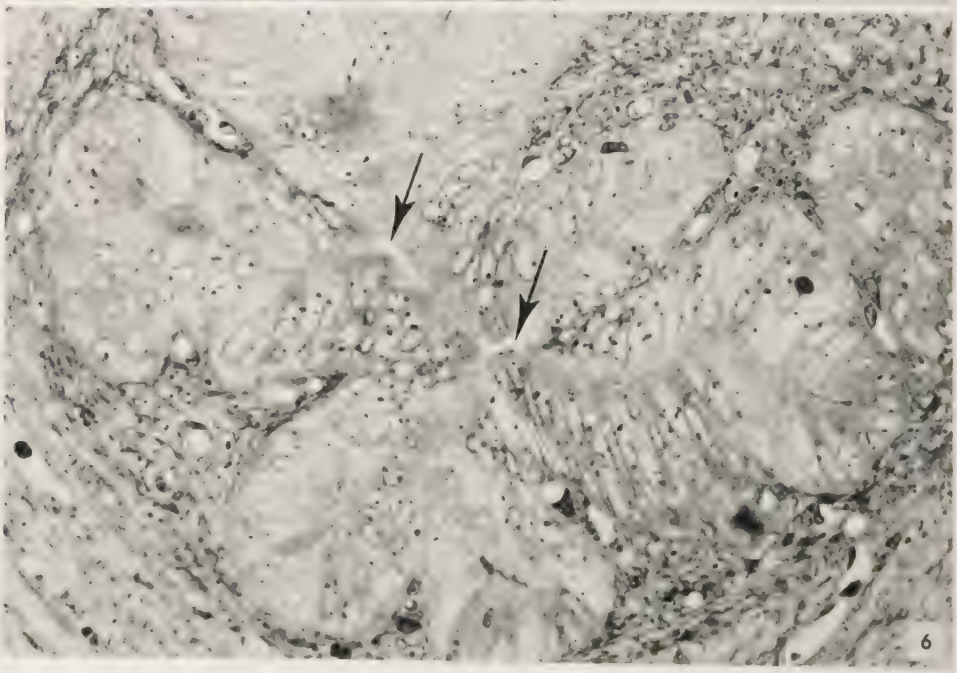

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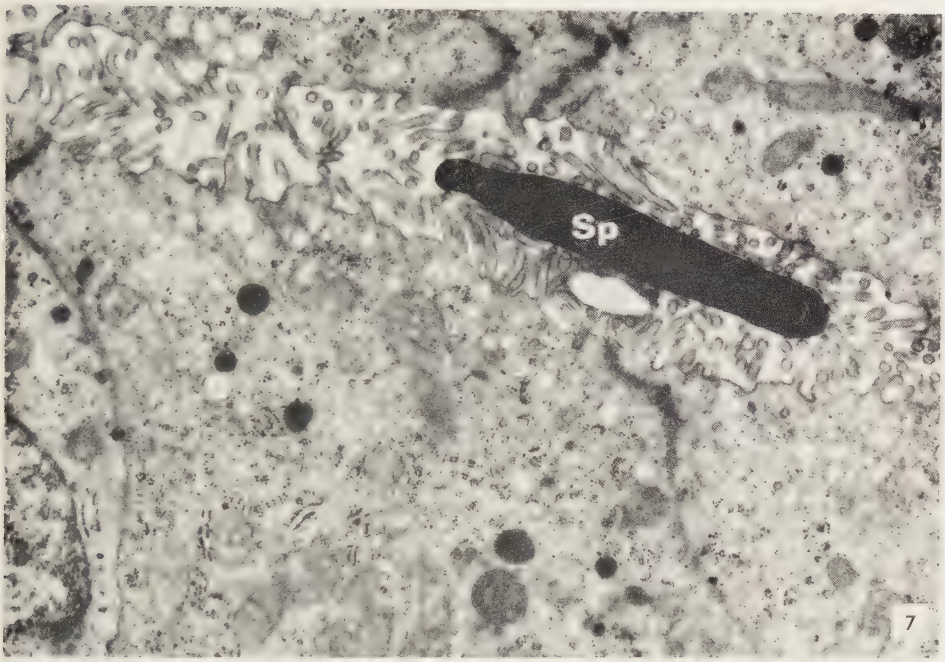
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III. OVUM AND ZYGOTE TRANSPORT

The methods used in studying the transport of the ova/zygotes in the oviducts of mammals are all unsatisfactory since the conditions in the living animal are disturbed (cf. Alden 1942, Blandau 1961, 1969). However, some methods are less disturbing than others, i.e. *in situ* or *in vivo* studies of the oviduct avoiding manipulation of the oviduct or its accessory membranes (cf. Blandau 1969).

Conventional morphological and experimental techniques have been used (Sobotta 1895, 1917, Westman 1926, Andersen 1927, Lewis and Wright 1935, Alden 1942, Burdick et al. 1942, Black and Asdell 1958, 1959, Clewe and Mastroianni 1958, Noyes et al. 1959, Benett and Rowson 1961, Blandau 1961 and 1969, Greenwald 1961, Harper 1961a,b, Humphrey 1968a and others), but are disadvantageous since the ova/zygotes lose their initial position and the structural relations are lost (cf. Longley and Black 1968, Reinius 1969a). Conventional histological techniques have also had the disadvantage of shrinkage and distortion of the tissues, which further changes the position of the ova/zygotes. An indication of the inadequacy of the histological techniques is the almost total lack of ova/zygotes in the preampulla and the intramural oviduct.

The use of a new morphological technique including rapid fixation by aortic perfusion and embedding of large specimens in plastic, and methods for their sectioning have made it possible to preserve the structural relationship in the oviduct and the uterus of the mouse with gametes and zygotes *in situ* (Reinius 1965, 1967a,b, 1969a,b). This technique probably leaves the tissues in conditions almost similar to those in the living animal and would thus seem valuable in studies of the ovum/zygote transport within the oviduct.

The aim of this report is to describe in detail the relationships between the ova/zygotes and the oviduct epithelium in the various segments of the oviduct observed with this new technique.

MATERIAL and METHODS

Males and virgin females of the CBA mouse strain were conditioned to artificial light from 05 to 19 hr. Pregnant females were obtained by pairing one male with two females and were selected by the presence of a vaginal plug. Day 1 was the day when the vaginal plug was found. The mouse material constitutes the main part (64 animals) of the series used for studies of the oviductal epithelium and oviductal ova and zygotes. The mouse material used followed similar patterns of age, stage and location of the ova/zygotes as other mouse strains (cf. Reinius 1969c).

Fixation was performed by aortic perfusion with 2.5% glutaraldehyde in Sørensen phosphate buffer, pH 7.3, osmolality about 450 mOsm.

The genital organs were dissected out and from each animal one oviduct was divided into four segments: the preampulla, the ampulla, the isthmus, and the junctura with the attached upper part of the uterine horn. The other oviduct with the ovary and upper part of the uterine horn was kept undivided in order to control possible traumatic effects of the fine dissection. Postfixation was performed in 1% osmium tetroxide in the Sørensen buffer, dehydration in ethanol, and embedding in Epon.

Sectioning for light microscopy was performed on a Porter-Blum ultramicrotome. Two to four μ thick plastic sections, covering an area up to 4 x 7mm and with ova/zygotes lying in situ in the different segments of the oviduct, and stained in Toluidine blue, were obtained according to a technique described earlier (Reinius 1969a). For electron microscopy large areas, covering about half the cross section of the oviduct, were sectioned on an LKB Ultrotome.

For further details of the technique,see Reinius (1969a).

RESULTS

According to the ultrastructure and distribution of the nonciliated cells the oviduct of the mouse has been divided into four segments (Reinius 1969a): the preampulla, the ampulla, the isthmus and the junctura.

The preampulla comprised the first loop of the oviduct. The muscular wall was thin and consisted of only two or three layers of cells. High, longitudinal epithelial folds lay tightly, filling the lumen. The epithelium consisted mainly of ciliated cells (Fig.2). The lumen was narrow with the cilia of the cells mostly meeting those of the opposing folds.

One ovum was found in the preampulla at Day 1/08hr (Figs.1 and 2).

The epithelial folds were separated slightly more than the diameter of the ovum along a distance occupied by a deformed and elongated follicle cell mass. The ovum was slightly elongated with the smaller diameter occupying the width of the lumen. The cilia of the ciliated cells lay almost in contact with the ovum and the follicle cell mass. There was no significant widening of the outer diameter of the preampullary loop.

The ampulla comprises the second loop. When containing the ova it was distended in spool shape (Fig.3). The muscular wall was even thinner than that of the preampulla and contained about two layers of cells. The epithelial folds were lower and more spaced. The epithelium consisted mainly of nonciliated cells, but ciliated cells were of frequent occurrence and were relatively more numerous towards the preampulla. The lumen was wide and filled with fluid and ova.

Unfertilized and fertilized ova were always found lying freely in the lumen at the ampullary-isthmic junction at Day 1/05 to 19 hr (Fig.3). They were still surrounded by more or less dispersed follicle cells 13hr after the sperm penetration.

The ampullary-isthmic junction was seen, when ova lay in the ampulla, where the outer diameter of the ampulla became narrow, the muscular wall was thicker and the lumen was filled by epithelial folds (Fig.3). The muscular cells were mainly circularly arranged, thus forming a sphincter-like structure. The epithelium of the folds consisted mainly of nonciliated cells. Stages when ova were passing this junction were not studied.

The isthmus comprises the third to the seventh or eighth loop of the oviduct. The muscular wall was thick, measuring about 20 to 30 μ and consisting mainly of circularly arranged cells. Epithelial folds were scanty, but a few longitudinal folds occurred in the loop next to the ampulla. In the rest of the isthmus circular folds were formed (Fig.4). The epithelium was mainly nonciliated, but a few ciliated cells were located in the grooves of the epithelial folds. The lumen was wide in the loops containing zygotes, but narrow, with apposing epithelial surfaces often meeting, in those devoid of zygotes.

The zygotes, normally at the 2-cell stage, lay freely in fluid in dilated loops, not occupying more than two neighbouring loops at Day 2/08 to 17hr (Fig.4). In a stereomicroscope during fine dissection the zygotes were observed to be freely movable in the wide luminal spaces.

The *junctura* constitutes the last extramural loop of the oviduct and the intramural oviduct with the colliculus tubarius. The muscular wall was thick, and consisted of mainly circularly arranged cells that lay close to the basement membrane of the epithelium (Fig.5). Only two or three layers of tightly packed connective tissue cells separated them. A thin external layer of longitudinally running muscle cells had direct continuity with the external uterine muscle layer. High, longitudinal epithelial folds filled the lumen but were not present in the colliculus (Fig.6). The epithelium consisted of only nonciliated cells. The lumen was narrow; the microvilli of the opposing epithelial cells met or lay a few microns apart.

The zygotes at the 5- to 8-cell stage lay singly or in a row, mainly extramurally, at Day 3/08 to 20hr, but in three animals, at Day 3/17hr, zygotes lay in that intramural part where longitudinal folds were present (Figs.5 and 6). The epithelium formed close contact with the almost spherical zygotes both extra- and intramurally; the microvilli lay in contact with the zona pellucida (Fig.7).

DISCUSSION

The exact times when the ova/zygotes enter and leave a segment cannot be deduced from this study, since the fixation of the material was performed at only a few times primarily adapted to the study of epithelial secretions. Further the location of ova/zygotes varies in time within several hours. Therefore, the times that have been set for the ova/zygotes to enter and stay in the different segments have been estimated both from data obtained from other publications (Lewis and Wright 1935, Humphrey 1968) and the present data.

The technique of aortic perfusion fixation with glutaraldehyde has many advantages over other fixation techniques (cf. Reinius 1969a). The morphology seems to reveal a good correlation between the conditions in the living animal and the findings in the plastic sections. The factors of specific importance when studying the transport of ova/zygotes in the oviduct are the rapidity of fixation and preservation of structural relationships. These factors are fulfilled by the technique (cf. Reinius 1969a).

The rapidity of fixation is important when studying ovum/zygote transport, since muscular contractions and cilia beating may continue after the conditions of the living animal have been disturbed, and the position of the ova/zygotes can thus be changed (cf. Longley and Black 1969).

The difficulty in finding ova/zygotes in the preampulla and in the intramural part of the junctura by conventional histology and by flushing can be explained by these postmortal effects, but also by rapid transit of the ova/zygotes through these segments. Indeed,the transit through the preampulla is a matter of seconds (cf. Blandau 1961).

From the sequences of events at the perfusion procedure the rapidity of fixation can be estimated. The fixative reached the oviduct within 10 sec after the aorta was clamped. After 30 to 60 sec the tissues had hardened and turned yellow indicating completed fixation (cf.Maunsbach 1966, Hopwood 1967, Reinius 1969a).

The structural features that could be studied with this technique and which influence the transport are: 1) appearance of the muscular wall, 2) the presence of epithelial folds, 3) the presence of ciliated cells, 4) the luminal width, and 5) the relation between the ova/zygotes and the epithelium. For each segment these features will be discussed with regard to their influence on the tubal transport. Only the mouse and the rat will be considered unless complementary data from other animals are necessary. For reviews on tubal egg transport, see Blandau (1961, 1969), Austin (1963) and Noyes (1965).

The evaluation of the transport mechanisms agree with earlier studies on the mouse and the rat using conventional histological and experimental techniques (cf. Humphrey 1968a), except for the transport in the junctura. In addition, more specific details have been presented of the preampulla, since an ovum was found in this segment.

In the preampulla the muscular wall is thin, the epithelial folds are extensive, many ciliated cells occur, and the lumen is rather narrow and only slightly wider than the diameter of the ovum, when this is found in the preampulla (see Figs.1 and 2 which are unique in the sense that an ovum has never been demonstrated by histological methods in this segment of the oviduct of the mouse or the rat). The elongation and deformation of the follicle cell mass increase the area of contact with the mucosa. The beating of the cilia should then pull the follicle cell mass with the ovum towards the ampulla. Thus, in the preampulla, the transport of the ovum seems to be an effect of the beating ciliated cells. Muscular transport is of less importance, since the lumen is filled with epithelial folds and the muscular wall is thin. The ciliary transport and the involvement of the follicular cell mass are consistent with the concept of Noyes (1965) and Blandau 1969).

In the ampulla the muscular wall is thin, the epithelial folds are less extensive, ciliated cells occur but are fewer than the nonciliated cells, and the lumen is widely distended. The ova lie free in the lumen. Although the muscular layer is thin, contractions in the wall would influence the flow of the large amount of fluid and also the transport of the ova. Further, distension of the smooth muscle cells should stimulate to contraction according to the bulking reaction. Thus, in the ampulla both ciliary beating and muscular contractions could influence the transport of ova towards the ampullary-isthmic junction. This is consistent with previous experiments (Alden 1942, Burdick et al. 1942, Blandau 1961, 1969, Humphrey 1968a).

The ampullary - isthmic junction demonstrates a muscular sphincter and appears in the stereomicroscope as a distinct blanched segment of a few millimeters in length and has an increased adrenergic nerve supply (Alden 1942, Blandau 1961, 1969, Brundin 1968). The lumen is closed by epithelial folds. This junction probably has to widen by relaxation of the muscular sphincter in order to let the ova pass, but this widening need to be extensive since ova/zygotes can pass through lumina not wider than their own diameter (cf. in the preampulla and the junctura). The junction seems to play a significant role in the transport, as indicated by antifertility experiments (see Greenwald 1967, Humphrey 1968b and Humphrey and Martin 1968).

In the isthmus the muscular wall is thick, the epithelial folds are scanty, the epithelium consists of nonciliated cells and a few ciliated cells, located in epithelial grooves, and the lumen is relatively wide in the loops where the zygotes are lying, but otherwise narrow. The distension of the loops containing the zygotes and much fluid would stimulate smooth muscle cell contraction. Thus, muscular activity can be expected to transport the ova by contraction and dilatation of the loops. The present results are consistent with experimental studies of the transport (Alden 1942, Burdick et al. 1942, Humphrey 1968a).

In the junctura the muscular wall is thick and lies close to the epithelium. The latter forms longitudinal folds and consists of only nonciliated cells. The lumen is narrow, not wider than a few microns, seemingly closed to the passage of zygotes. The epithelium forms contact with the zygotes almost all around, leaving no or very little space for fluid. In

the perfused material the zygotes are spherical, but in frozen and cryostat sectioned material the zygotes are angular in shape (Reinius, unpublished). Rat zygotes fixed *in situ* in the segment by aortic perfusion are conical, with the top of the cone directed towards the uterus (Nilsson and Reinius 1969), and rat zygotes fixed by flushing from the junctura are also elongated (Schlafke and Enders 1967). It seems as if the shape of the zygote at this location depends upon the close relationship to the epithelium and the relative rigidity of the epithelium and the zygote at the moment when the fixative reaches them.

The zygotes lie in the extramural part of the junctura for many hours or up to 30hr, according to Humphrey (1968a). However, the transit through the intramural part has been described as a rush but also as a stay of up to 24hr (Burdick et al. 1942, Humphrey 1968a). In the present material this time has to be estimated from the finding of intramural zygotes which indicates that the passage should not be very rapid and could take several minutes or a few hours.

The transport of the zygotes through the junctura, both the extra- and the intramural parts, indicated by the present data, should be an effect of the muscular layer. Preliminarily, it should be a dilatation of the muscular layer and a widening of the lumen since the lumen is otherwise constantly closed. This dilatation, however, is probably not extensive but very local near the zygotes and will concern mainly the muscle cells close to the epithelial membrane. The transport would then occur by slow waves of contraction and dilatation, that push or squeeze the zygotes through the junctura in intimate contact with the epithelium and is similar both in the extra- and intramural parts of the junctura.

In the intramural part of the human oviduct a type of organization of the muscular layers has been described that indicates contraction waves, which are preceded by dilatation of the lumen (Vasen 1959). Further, in man, the bat, *Glossophaga soricina*, the rat, and in the guinea-pig the lumen is narrow similarly to that in the mouse (Nilsson and Reinius 1969, and unpublished). Thus, a similar mechanism of zygote transport may exist in other mammals.

This mechanism is not consistent with a peristaltic rush that forces fluid and the zygotes through the segment into the uterus (cf. Humphrey 1968a).

Retention of zygotes in the junctura has been described as an effect

of the administration of ovarian hormones (Whitney and Burdick 1936) although it does not occur at the same great extent as the retention at the ampullary-isthmic junction (Greenwald 1967, Humphrey 1968b, Humphrey and Martin 1968).

The significance of the narrow lumen in the junctura for the transport of zygotes is not clear. It would seem disadvantageous for the transport, but rather a selection device for a few actively migrating spermatozoa to enter the oviduct and to separate two different environments, that of the oviduct and that of the uterus (Reinius 1969a,b).

SUMMARY

Mouse oviducts with ova/zygotes *in situ* were examined by light and electron microscopy after fixation by aortic perfusion with glutaraldehyde, postfixation in osmium tetroxide and embedding in Epon. This technique gives accurate data for the evalution of the transport mechanisms in the oviduct.

The transport of the ova/zygotes through the oviduct is considered to be as follows. Through the preampulla the transport is an effect mainly of the ciliary beating; through the ampulla an effect of both ciliary beating and muscular contractions. The ova are kept for a longer period at the ampullary-isthmic junction, which is closed by epithelial folds and a sphincter-like structure. In the isthmus the transport of the zygotes is due to muscular contraction and dilatation of the loops containing much fluid around the zygotes. In the junctura, consisting of the intramural oviduct and the proximate extramural loop, the transport is an effect of muscular contraction and dilatation occurring locally around the zygotes, which pushes or squeezes them slowly through a narrow lumen.

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LEGENDS

Fig. 1. Preampulla with an unfertilized ovum, Day 1/08hr. Note the deformation of the follicle cell mass and the epithelial folds, which have only separated slightly more than the diameter of the ovum. Light microscopy x 160.

Fig. 2. Preampulla with an unfertilized ovum, Day 1/08hr. The same ovum as in fig. 1. The epithelium consists mainly of ciliated cells (Cc); the cilia are indicated by arrows. Few nonciliated cells (Nc) occur. Follicle cells (Fc); the matrix of the follicle cell mass is not visualized; zona pellucida (Zp). Light microscopy x 700.

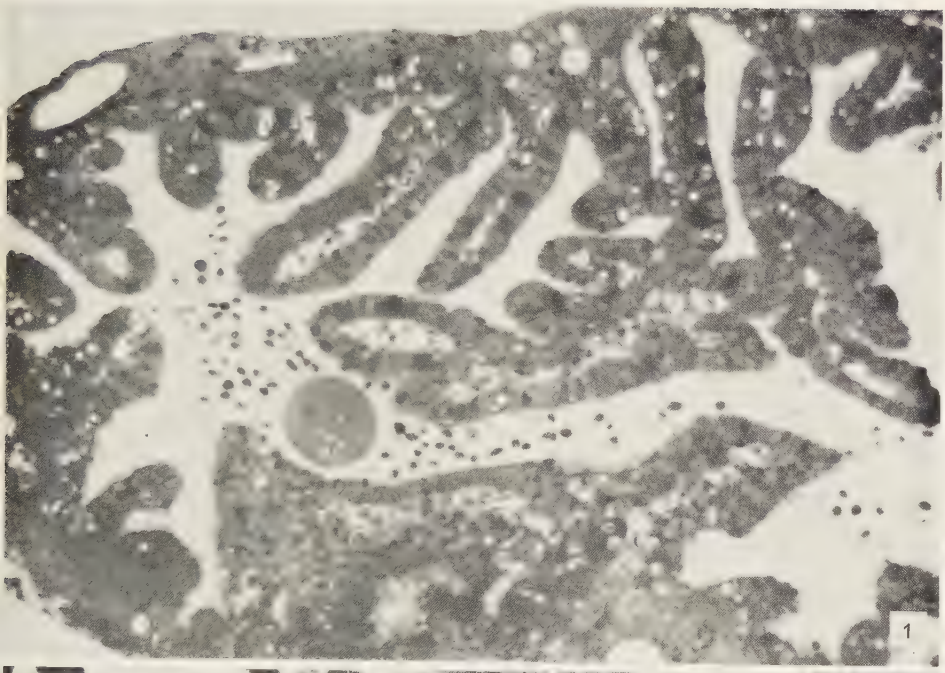
Fig. 3. Ampulla with a pronuclear ovum, Day 1/17hr. The ovum, surrounded by follicle cells, lies in the dilated part of the ampulla with the ampullary-isthmic junction to the right. The muscular wall of the ampulla is thin; the epithelial cells are mainly nonciliated. At the ampullary--isthmic junction, the epithelium forms high folds protruding into the ampullary lumen and occluding the lumen (arrow) of the junction. The muscular wall of the junction is thick. Light microscopy x 150.

Fig. 4. Isthmus with two 2-cell zygotes, Day 2/16hr. The muscular wall is thick. The epithelium, which consists mainly of nonciliated cells, is arranged in circular folds. The zygotes lie free in the rather wide lumen. Light microscopy x 160.

Fig. 5. Junctura at the border between the extramural and the intramural part with a 5-cell zygote, Day 3/17hr. The muscular wall is thick and lies close to the epithelial basement membrane (arrows). The epithelial folds run longitudinally and consist of only nonciliated cells. The epithelium lies in close contact with the zygote. Light microscopy x 150.

Fig. 6. Junctura with a 5-cell zygote in the intramural part, Day 3/17hr. The zygote lies where the epithelial folds disappear in the colliculus tubarius. The uterine opening of the oviduct is indicated by an arrow. Light microscopy x 160.

Fig. 7. Junctura, the extramural part, with a 6-cell zygote, Day 3/16hr. The close contact between the zygote and the epithelium is seen; the microvilli (Mv) of the epithelium lie in direct contact with the zona pellucida (Zp). Blastomere (B); perivitelline space (Ps). Electron microscopy x 6.500.



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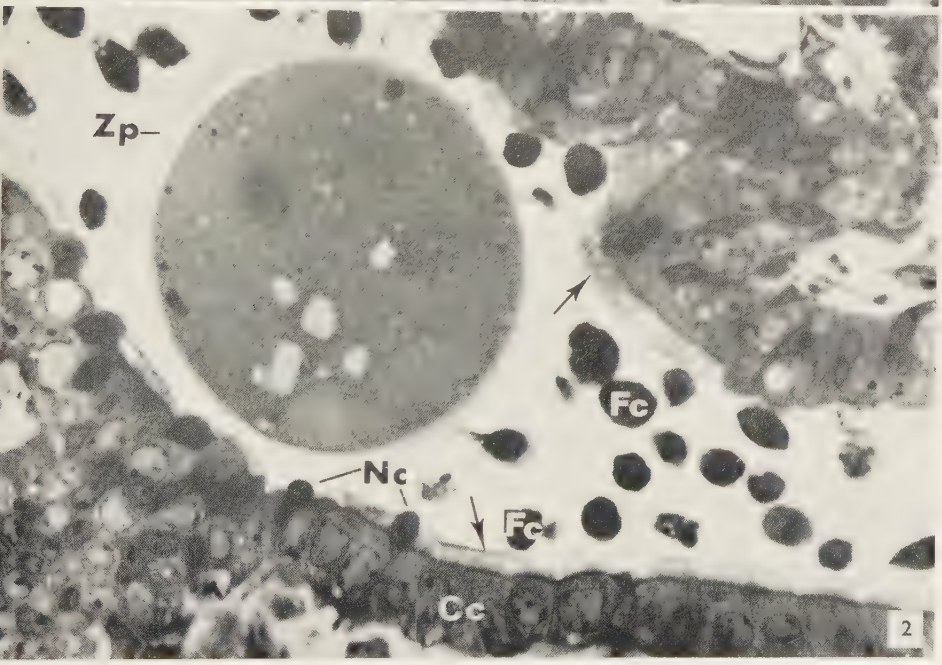

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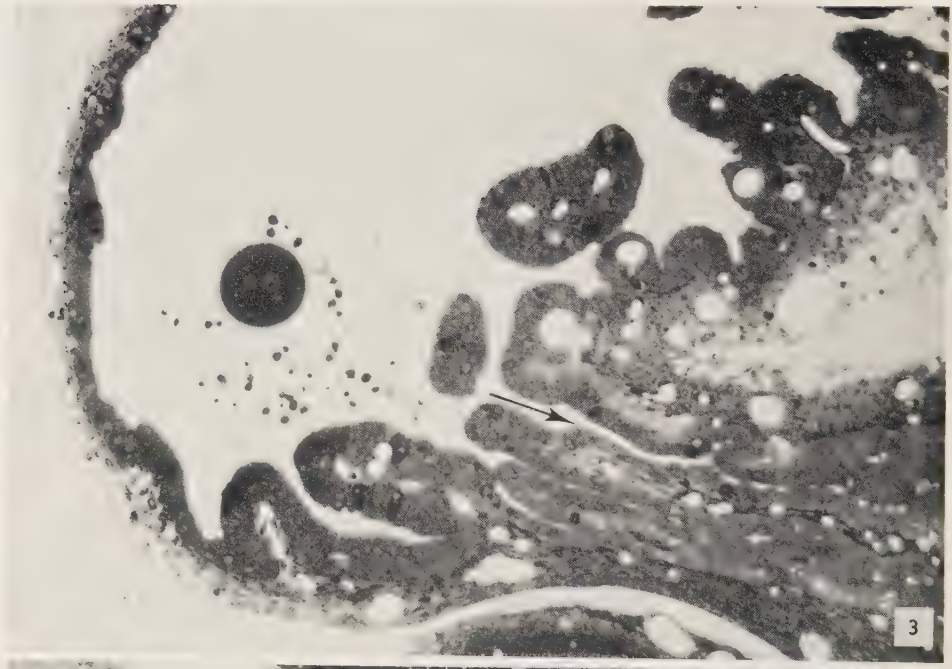
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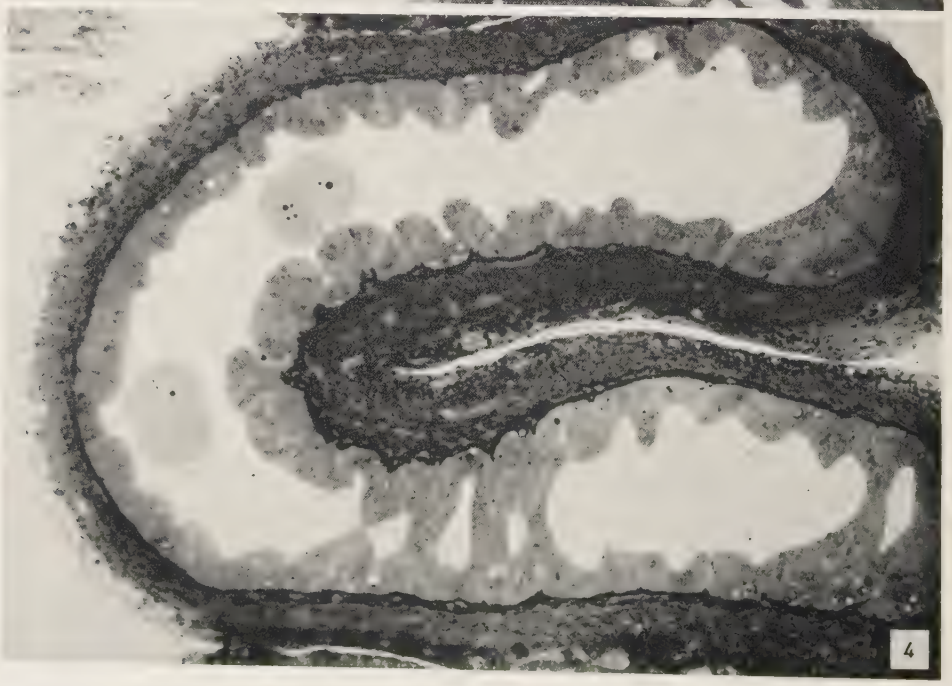
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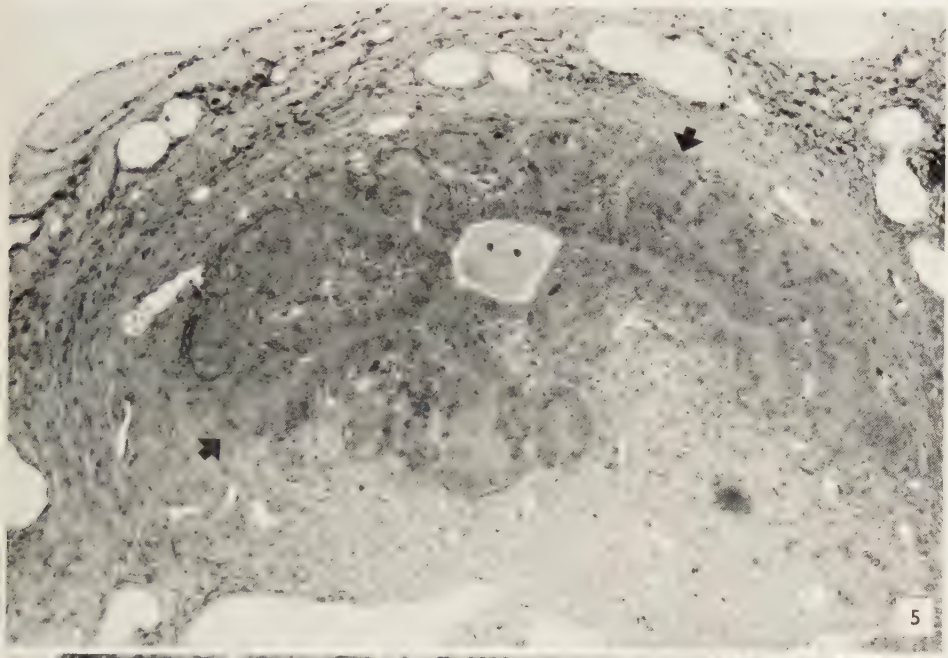

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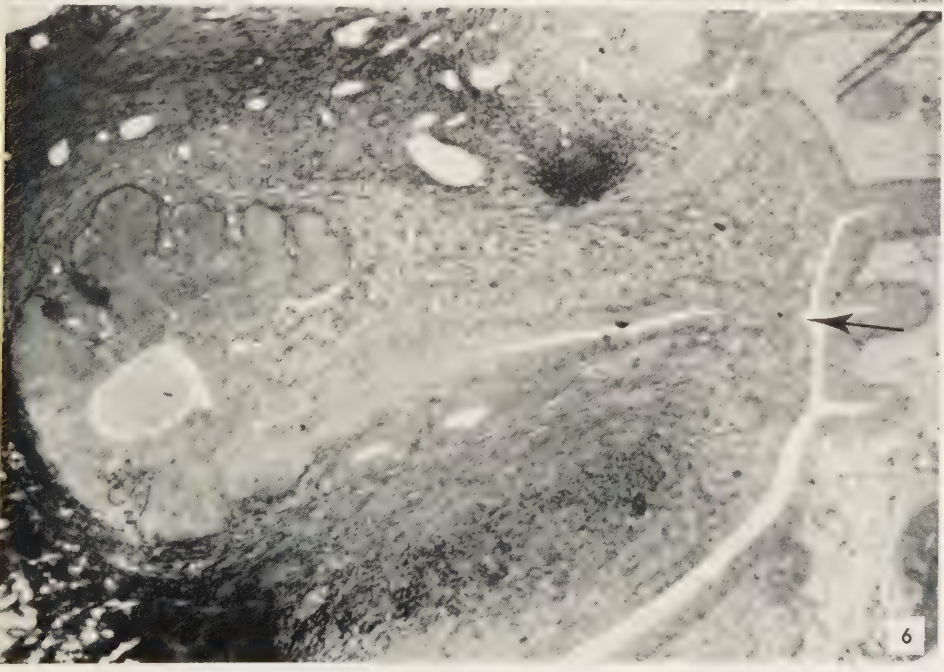

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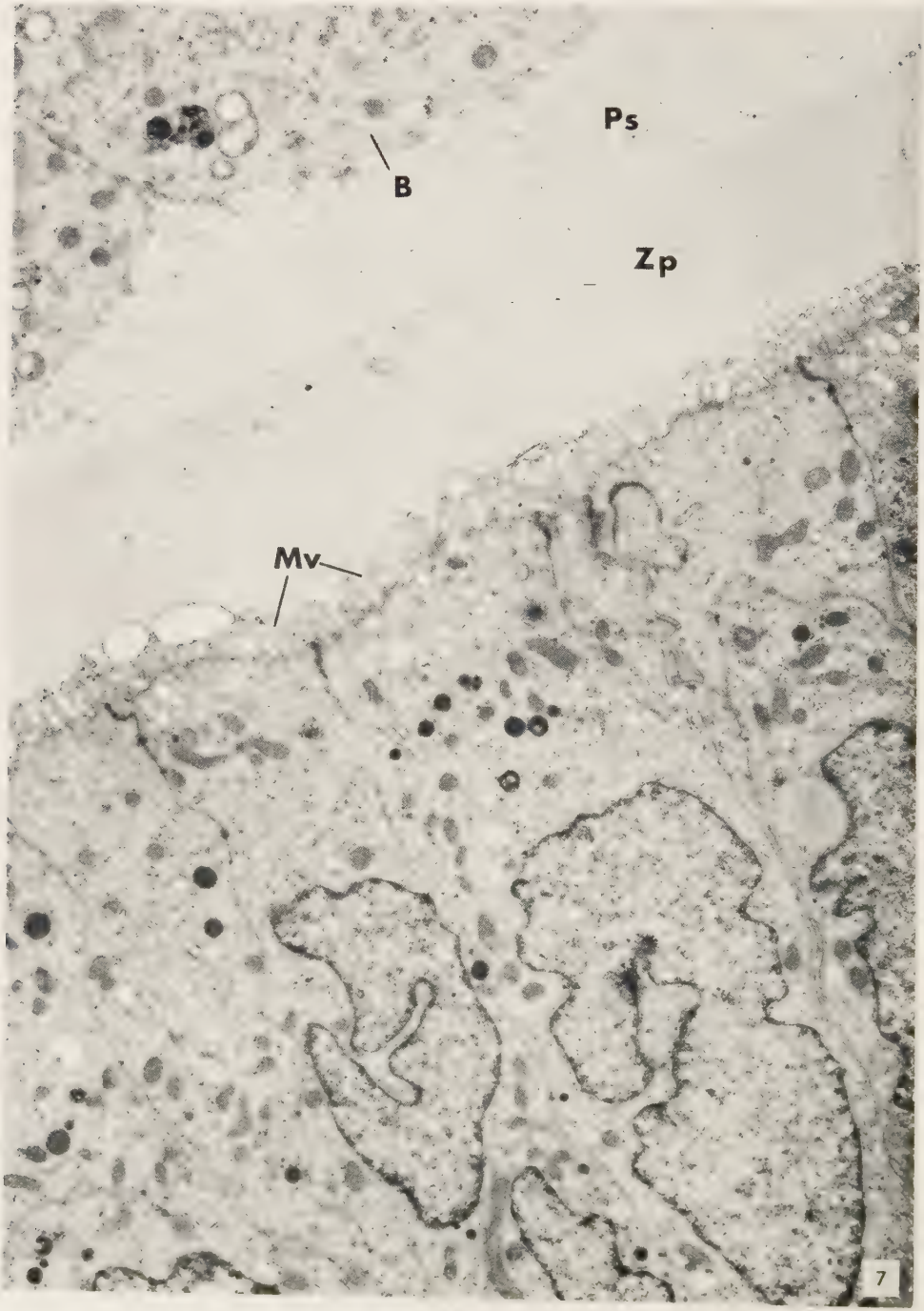
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IV. OVUM AND ZYGOTE DEVELOPMENT

Ultrastructural studies of the oviductal epithelium of the mouse indicate different compositions of the secretion in the ampulla, the isthmus and the junctura, in which the zygotes have reached different stages of development (Reinius 1967a, 1969a). The secretion of the oviduct and the metabolism of the zygote are two components of an integrated system (Biggers et al. 1967, Restall 1967). In order to understand the significance of the secretion in the different segments of the oviduct, one has therefore to consider also the metabolism of the zygote and the local relationship between the epithelium and the zygote.

Indirect results from *in vitro* culture experiments have given important information about the metabolism of the zygote of the mouse (Whitten 1957, Mintz 1964, 1965, Brinster 1963, 1965 a-c, 1967a, 1969, Izquierdo and Roblero 1965, Brinster and Thomson 1966, Biggers et al.1967, Mills and Brinster 1967, Monesi and Salfi 1967, Whittingham and Biggers 1967, Wales and Biggers 1968, Wales and Brinster 1968, Whittingham 1968,and others). Further, biochemical and cytochemical studies have been used on ova and zygotes of the mouse and the rat in culture media to elucidate the type of metabolism at different stages of development (Gresson 1941, Dalcq 1951a,b, 1957, Austin 1953, Austin and Braden 1953, Braden and Austin 1953, Izquierdo 1954, Austin and Bishop 1959, Mulnard 1961, Thomson and Brinster 1966, Stern and Biggers 1968,and others; see also Hafez and Sugavara 1969). However, the metabolism of the zygote may not be identical *in vivo and in vitro*. More direct information about the metabolism of the zygote and the relationship between the epithelium and the zygote has been obtained by studies of amino acid and protein transfer to the zygote, RNA and protein contents and enzyme activity of the zygote (Alfert 1950, Flax 1953, Augustin and Moser 1955, Greenwald and Everett 1959, Glass 1963, Weitlauf and Greenwald 1965, 1967).

The ultrastructural pattern of various organelles indicates the type of metabolic activities of the ovum/zygote. Such studies have been perfor-

med, although various dissection and flushing techniques may have altered the natural relationship between the ova/zygotes and the epithelium. The rat has been examined by various authors i.e. Sotelo and Porter (1959), Izquierdo and Vial (1962), Mazanek and Dvořák (1963), Mazanek (1965), Enders and Schlafke (1965), Szollosi (1966), and Schlafke and Enders (1967), and are only relevant for this animal. Preliminary results are also available for the mouse (Enders and Schlafke 1965, Hillman et al. 1967, Sackler 1968).

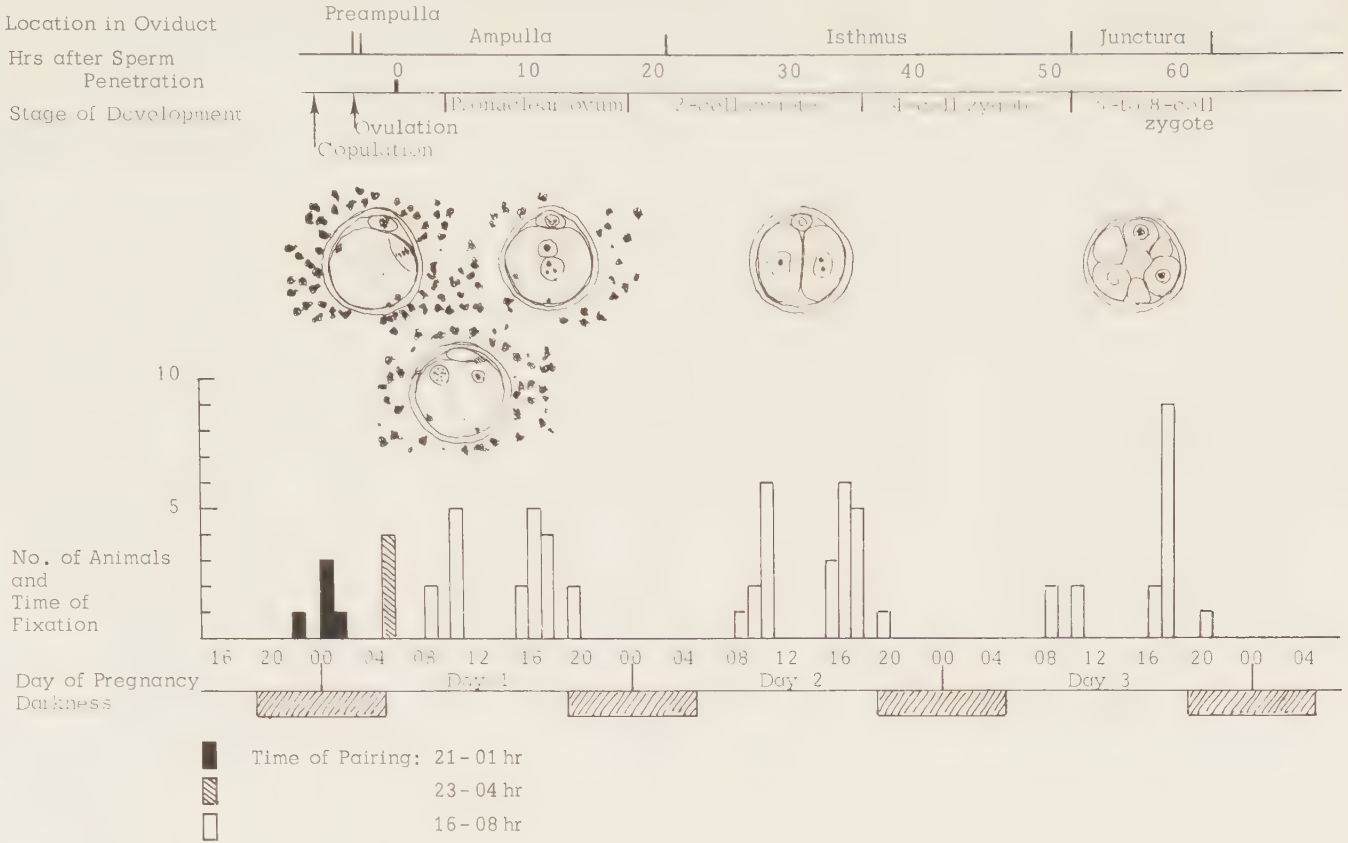
A direct technique has been developed that makes it possible to study with the light and electron microscope the whole oviduct of the mouse with gametes and zygotes in situ (Reinius 1967a, 1969a-c). The aim of this report is therefore to describe the ultrastructure of the ovum and the developing zygote in situ in order to elucidate the metabolism of the zygote and the relationship of the epithelium and the zygote in the oviduct of the mouse. This may give further information on the significance of secretion in the different segments of the oviduct.

MATERIAL AND METHODS

The material comprised the same animals as used for studies of the oviductal epithelium (Reinius 1969a), see Table I. Males and virgin females of the CBA mouse strain were used and conditioned to artificial light from 05 to 19 hr. For mating, two females were left with one male overnight (about 16 to 09 hr) or for shorter periods during the night. Pregnancy was determined by the presence of a vaginal plug. Day 1 was the day when the vaginal plug was found. The animals (64 mice) were fixed at the following stages and times: unfertilized ova lying in the preampulla or ampulla at Day 1/05 to 08 hr, pronuclear ova lying in the ampulla at Day 1/10 hr to 17 hr, 2-cell zygotes lying in the isthmus at Day 2/09 to 17 hr, and 5 to 8-cell zygotes lying in the junctura at Day 3/08 to 20 hr.

The various times of the events were deduced and set as follows. Copulation should occur at about midnight on Day 1, 3 hr before ovulation (cf. Snell et al. 1940). Ovulation is normally controlled by the diurnal rhythm of light and darkness (cf. Snell et al. 1940, Braden and Austin 1954, Braden 1957, Whitten and Dagg 1961), and in the present material should have taken place at about Day 1/03 hr, 3 hr after the midpoint of the dark phase. Penetration, synonymous with the spermato-

Table I. Data of animals with gametes and zygotes in the oviduct



zoon penetration of the plasma membrane of the ovum, was estimated to occur at Day 1/06, 3 hr after the approximate time of ovulation and 4 hr before the development of the pronucleus (cf. Lewis and Wright 1935, Braden and Austin 1954, Edwards and Gates 1959). The first cleavage should occur at Day 2/00 hr (cf. Sobotta 1895, Long and Mark 1911, Lewis and Wright 1935, Gresson 1941, Whitten and Dagg 1961). The second cleavage would occur at about Day 2/18 hr, i.e. 18 hr after the first cleavage (cf. Long and Mark 1911, Whitten and Dagg 1961). The third cleavage was observed at about Day 3/10 hr or approximately 55 hr after ovulation (cf. Sobotta 1895, Long and Mark 1911, Lewis and Wright 1935, Whitten and Dagg 1961).

Fixation was performed by aortic perfusion with 2.5% glutaraldehyde in Sørensen phosphate buffer, pH 7.3, osmolality about 450 milliosmoles. The genital organs were dissected out and the oviduct was divided into the four segments, the preampulla, ampulla, isthmus, and junctura (Reinius 1969a). Postfixation was performed in 1% osmium tetroxide in the Sørensen buffer, and embedding in Epon. Sections 5 to 10μ thick of segments containing ova/zygotes were cut on a Porter Blum microtome. The sections were discarded until they were seen to contain ova/zygotes in the stereo-microscope of the microtome, after which one or two ova or zygotes were sectioned serially about 2 to 4μ thick for light microscopy and stained with 1% Toluidine blue in 1% borax or 1% sodium bicarbonate (Pease 1964, Reinius 1966). For electron microscopy thin sections of one or two ova or zygotes from each animal were cut on an LKB Ultrotome. The sections, which were cut gray to yellow, were picked up on one-hole grids (Galey and Nilsson 1966), and contrast stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963). An RCA EMU 3B electron microscope was used at an accelarating voltage of 50 KV.

For further details of the fixation, preparation and sectioning see Reinius(1969a).

RESULTS

Unfertilized Ovum

Only one ovum was found in the preampulla (Reinius 1969c). All the other newly discharged ova lay in the distended ampulla, often clumped together by the follicle cells to form a large mass.

The follicle cells were loosely arranged in about ten layers of cells around the ovum (Fig.1). They lacked cytoplasmic projections into the zona pellucida. The zona pellucida closely outlined the ovum and first polar body. The zona measured about 3.5μ. It consisted of a thick homogeneous and structureless or fine fibrillar inner part and a thinner and more loose outer part (Fig.3). Occasionally vesicles or cytoplasmic remnants were found in the zona. The perivitelline space, into which the microvilli of the ovum extended, was narrow, about 0.2 to 0.4μ. Around the polar body there was a wide perivitelline space, where cellular debris was often present.

The ovum was almost spherical with only a slight flattening at the polar body side, and measured on the average 88μ in diameter. The total diameter of the ovum with the zona pellucida measured about 95μ.

The ultrastructure of many cellular components did not differ in the unfertilized and the fertilized ova, and therefore some of the figures referred to below apply also to the fertilized ovum.

The ovum possessed a moderate number of regularly distributed microvilli, 0.2 to 0.5μ tall, mostly bent tangentially to the vitelline surface (Figs.2 and 3).

The chromosomes of the second maturation spindle were seen on one side of the cell (Figs.1 and 3). The chromosomes had a fine fibrillar or granular appearance. The area around the chromosomes was seen as a less electron dense area lacking cytoplasmic components except for the tubular system of the spindle. Around this area many vesicles were seen, 0.2 to 0.3μ in diameter. The plasma membrane over the spindle was smooth, tightly pressed against the zona pellucida and had an electron dense inner borderline (Fig.3) as described by Szollosi (1967).

The mitochondria lay singly or in small groups, scattered all over the cytoplasm. They were mostly spherical, measuring 0.5 to 0.7μ in diameter (Figs.3,8 and 9). The cristae were sparse and arranged peripherally, parallel with the outer membranes, and the centre of the mitochondrion appeared vacuolized. The Golgi complex consisted of small aggregates of parallel membranes and a few vesicles (Fig.8). The aggregates were few and scattered around the whole cytoplasm and were not found to be associated with other specific cytoplasmic components. The endoplasmic reticulum lacked ribosomes (Figs.6 and 8). Smooth vesicles,

0.2 to 0.3μ in diameter, were distributed throughout the cytoplasm, and specially concentrated around the second maturation spindle. Free ribosomes were sparse in the cytoplasm.

The yolk nucleus (Anderson and Beams 1960, Weakley 1967), consisted of several complexes; each complex was slightly oval and measured up to 4.5 to 5.0μ in diameter (Figs.1,2, 4 and 5). In a median section of the ovum, between one and four of the complexes were seen in the periphery of the ovum. They had an inner electron dense nucleus surrounded by a thin halo of a less electron dense cytoplasm. Around this a large mass of mainly small spherical vesicles, 0.1 to 0.2μ in diameter, and some multivesicular bodies and elongated vesicles were seen, all lying in a slightly electron dense matrix. Some vesicles had an inner electron dense structure. No membrane was formed around the complex, although it had a rather smooth outer surface. The vesicles with a denser inner structure were similar to the cortical granules.

The cortical granules (Szollosi 1962, Austin 1965), lay just beneath the plasma membrane, at certain sites almost touching it (Fig.2). The granules were mostly spherical and measured 0.2 to 0.4μ in diameter, but some of them were elongated. Their inner structure was electron dense and mostly filled the granules entirely. Other granules had a peripheral light rim and hence a distinguishable enveloping membrane.

Fibrous elements with dark bands of a periodicity of 300 to 320Å, about the same breadth as the periodicity and of some microns in length, occurred abundantly in small stacks scattered all over the cytoplasm (Figs.2,5 and 8). Lipid granules about 0.9 to 1.2μ in diameter were relatively frequent and arranged in groups, most abundantly in the central region of the ovum (Fig.8). Granules with a homogeneous structure surrounded by a single membrane and measuring about 0.5 to 0.6μ in diameter were seen in small groups near either the complexes of the yolk nucleus or the mitochondrial aggregates. A few granules had a dense body up to 0.1μ in diameter localized at the membrane. Myelin-like figures were rarely seen.

The first polar body varied widely in shape and structure. It was mostly oval and could be as large as up to 20μ in diameter; it had no nucleus but contained a chromatine aggregate. The cytoplasm often was similar to that of the ovum or could be either very dense and structureless or loose

and consisting of mostly vesicles. No ova were found to lack the first polar body at this stage, neither were there any signs of polar body extrusion.

Pronuclear Ovum

The fertilized or pronuclear ovum was still localized in the distended ampulla near the ampullary-isthmic junction about 4 to 13 hr after the sperm penetration (Fig.4). The ova lay more separately and the follicle cells were more loosely arranged. The follicle cells formed about two to five layers of cells around the ovum and lacked cytoplasmic connection with it. Spermatozoa were occasionally seen among the cells and at the zona pellucida.

The zona pellucida showed a similar structure to that of the unfertilized ovum, with a loose outer and a more compact inner zone. However, when fertilized ova were successfully sectioned a slit in the membrane, about the breadth of the spermatozoa head (1.0μ), was found. The slit ran obliquely through the zona and was occasionally seen to contain a spermatozoon tail (cf. Dickmann 1964).

The perivitelline space was still narrow, as in the unfertilized ovum. Slightly more debris was found in the space, especially around the polar bodies, where the space also was wide. Normally, parts of the tail of a spermatozoon and rarely a supernumerous head were seen in the perivitelline space.

The diameter of the fertilized ovum had diminished and now measured about 81μ. The total diameter with the zona pellucida measured about 88μ.

The plasma membrane did not differ markedly from that of the unfertilized ovum.

The pronuclei were located in the middle of the cell (Fig.4). The larger - the male pronucleus - (Austin and Braden 1955, Edwards and Sirlin 1956) was up to 20μ in diameter. The smaller - the female pronucleus - measured about 12 to 15μ in diameter and was often localized at the polar body side. No difference in the ultrastructure of the two pronuclei was observed. The pronuclear membranes were slightly folded; the outer membrane bulged from the inner at many places and small spaces were formed in between the two membranes (Fig.6). These spaces were often seen to contain fine fibrillar or granular structures about 0.1μ in diameter (cf. Szollosi 1965b). The outer membrane lay in contact with or formed part of

vesicles about 0.2 to 0.3μ in diameter. In some ova the inner pronuclear membrane was smooth, and the outer membrane was folded. Nuclear pores were seen but to a fairly small extent. The nucleoplasm was homogeneous and had a slightly electron dense appearance (Figs.6 and 7). The nucleoli lay in the centre of the pronucleus, one large or two or three smaller ones. They were spherical with a smooth outer surface, and a single nucleolus could measure up to 8 to 8.5μ in diameter in the male pronuclei and up to 7μ in the female. The nucleolus had an electron dense appearance and consisted of a rather coarse and tightly packed network of fibrils and/or granules less than 100Å in diameter (Fig.7). Granules with a diameter of 300 to 400Å were seen in groups forming up to 0.6μ large spherical structures free in the nucleoplasm.

The cytoplasmic organelles and inclusions had the same organisation and structure as in the unfertilized ovum except for the mitochondria and granules,which were more concentrated around the pronuclei (Fig.4). The cortical granules were lacking. Spermatozoon remnants as well as parts of the middle piece with filaments and disarranged mitochondria were seen (Fig.9). No membranes surrounded the mid-piece.

The first polar body had normally disappeared. The second polar body was large, about 10μ in diameter and had a nucleus. Often a cytoplasmic bridge was formed between the polar body and the cytoplasm of the ovum. The outer cell membrane of the second polar body was relatively smooth with a few short microvilli. The cytoplasm was similar to that of the ovum but lacked complexes of the yolk nucleus.

Two - Cell Zygote

The 2-cell zygotes usually lay grouped in the middle loops of the isthmus 26 to 35 hr after sperm penetration. The lumen of the isthmus was rather wide and the zygotes lay freely in the tubal fluid. The follicle cells had disappeared from the zygotes.

The zona pellucida had not changed markedly in structure and was formed after the shape of the two cells and the second polar body (Fig.10). The perivitelline space was the same, about 0.2 to 0.4μ wide and only became wider at the junctions of the cells. Often cellular debris and parts of the sperm tail and occasionally the head of a supernumerous sperm were found in this space.

The two cells - the blastomeres - had almost the same size and shape (Fig.10). The cells were flattened against each other. Each cell measured about 73μ x 45μ. The 2-cell zygote with the zona pellucida measured about 98 x 80μ. The blastomeres had a moderate number of microvilli on the free surfaces and also on the cell surfaces adjoining the two blastomeres. These adjoining surfaces lay at a mean distance of 0.1 to 0.2μ apart, but the microvilli or broad cytoplasmic projections often intermingled or lay in contact with the apposing surfaces (Fig. 12). No tight junctions or cytoplasmic bridges were observed.

The nucleus was placed in the centre of the blastomere. The nuclei of the two cells had the same structure and form. The nucleus was oval or of irregular shape, it had one longer axis, parallel to the long axis of the cell, and measured about 20μ. The nuclear membranes were slightly folded (Fig.11). Nuclear pores were more frequent than in the pronucleus. Wide spaces between the outer and inner membranes containing granules were rarely seen. Intranuclear annulate lamellae (IAL) were seen frequently in the nucleus (Fig.12). They usually formed contacts with the inner nuclear membrane with blind endings and extended into the nucleus either ending freely or in contact with nucleoli. Some profiles appeared to be branched. The nucleoli, up to three to four of which were seen in one section of each nucleus, lay sometimes near the nuclear membrane. They were spherical and measured up to 9μ in diameter, and had the same structure as the nucleoli of the pronuclear ova. A few nucleoli showed irregular shapes that formed two or three different densities. Granular condensations were fairly frequent in the caryoplasm and were of two types (Fig. 11). The first type consisted of large granules 300 to 400Å in diameter in spherical aggregates similar to those of the pronuclei, and the other consisted of smaller granules 100 to 150Å in diameter which occurred in irregular formations often near the nucleoli or the nuclear membrane.

The cytoplasm was similar in the two blastomeres. The mitochondria were mainly spherical with vacuoles, but a few were slightly elongated and showed some transverse cristae. The aggregates of the Golgi complex were still small (Fig.12). The endoplasmic reticulum consisted of vesicles with a diameter of about 0.2 to 0.3μ attached to a few of which were one or two ribosomes. The amount of free ribosomes had not in-

creased significantly. The complexes of the yolk nucleus had split up further, so that they were smaller, up to 2 to 3μ, and more numerous (Fig.12). They were still located in the periphery of the cells. They lacked the nucleus of the electron dense material and consisted of spherical or elongated vesicles and multivesicular bodies in a dense substance. Fibrous elements and lipid granules occurred to the same extent and with the same distribution as in the one-cell stages. The homogeneous granules of 0.5μ were more clumped together in the 2-cell blastomere and often lay close to parts of the complexes of the yolk nucleus in the periphery of the blastomeres. Sperm tail filaments were observed rarely in the cytoplasm. The second polar body still remained, lying on one side just beneath the zona pellucida in the crest formed by the two blastomeres meeting each other. Often the two nuclear membranes of the polar body lay widely apart and the chromatine was clumped.

Five - to Eight - Cell Zygote

The 5- to 8-cell zygotes were located singly or in a group of two or more in the junctura of the oviduct either in the last extramural loop or intramurally, 50 to 62 hr after sperm penetration. The lumen of the junctura was normally so narrow that the zona of the zygote came into direct contact with the microvilli of the epithelium (Figs.13 and 14). The epithelium then formed almost spherical chambers around the zygote according to the shape of the latter.

The zona pellucida was homogeneous and structureless or fine fibrillar throughout and lacked an outer loosely arranged structure (Fig.14). It was about 3,5μ thick and was almost spherical around the zygote.

The perivitelline space varied greatly in width according to the unevenness between the different blastomeres. It was sometimes filled by cellular debris, fibrillar structures of a sperm tail and occasionally a head.

The 5- to 8-cell zygote was spherical or slightly elongated, its longest diameter with the zona measuring, on the average, 94μ (Fig.13). The 5- to 8-cell stage consisted of both undivided cells of the 4-cell zygote and/or cells of the 8-cell zygote. The 4-cell blastomeres measured up to 45 to 50μ, the 8-cell blastomeres 35 to 40μ. However, in the complete 8-cell zygote there was some variation in size of the cells. The cells were cone-like in shape, with the top of the cone directed into the

centre of the zygote and the base somewhat rounded with the convex side against the zona pellucida. All cells seemed to reach a free surface.

The microvilli of the plasma membrane were few and short. The borders between the cells were rather smooth, with only a few microvilli causing a slight separation of the membranes that otherwise lay close together (Fig.15). Interdigitations were also seen. No typical junctional complexes such as desmosomes were observed, but sometimes the intercellular membranes lay close together, almost forming tight junctions.

The nuclei were spherical, and located in the centre of the cells (Fig. 15). They measured in 4-cell blastomeres about 14μ in diameter and in the 8-cell blastomere about 12μ.

The nuclear membranes were similar to those in the 2-cell blastomere but did not form folds. The nuclear pores occurred in a larger number (Fig.16). The intranuclear annulate lamellae were frequently seen in the large 4-cell nuclei but were few in the 8-cell nuclei (Figs.15 and 16), and formed a tight connection with the nuclear membrane and/or the nucleolus.

Normally one nucleolus was seen in the centre of the nucleus. Centrally it had a spherical, fine fibrillar or granular structure similar to the nucleoli of earlier stages, but the periphery was irregularly formed by mainly larger granules and/or fibrils in a loose, net-like arrangement, the nucleolonema (Fig.17). These granules and/or fibrils measured about 100 to 150Å in diameter.

Cells in mitotic divisions were observed normally in the metaphase. An area in the centre of the cell was seen to be devoid of the large organelles but contained a few ribosomes, microtubuli and chromosomes of a fine fibrillar and/or granular structure. In the outer border of the light area small vesicles were concentrated.

Most mitochondria had a spherical shape and no inner structure, but a few were rod-shaped and had many transverse cristae and an electron dense matrix (Fig.18).

The Golgi apparatus formed a few small complexes scattered in the cytoplasm. The endoplasmic reticulum consisted of tubules and cisternae, with some ribosomes attached, and an increased number of free ribosomes (Fig.18). The tubules or cisternae lay freely in the cytoplasm but were often in direct contact with specific crystalloid inclusions (Figs. 15 and 18). The latter were square or rectangular in shape and consisted of 5 to

10 bundles, each of which measured 600Å broad and up to 1 to 2μ long and showed regular banding with a periodicity of about 130Å. When the crystals were cut in oblique angles, different bandings appeared. The fibrils and the lipid granules had not changed in structure or distribution and lay scattered all over the cytoplasm. Granules of 0.5μ occurred in large groups, mostly with a peripheral localization. An increased number of them had dense granules at the encircling membrane.

Myelin-like figures and vacuoles, containing irregularly shaped electron dense material, were seen in the blastomeres and were often located on one side in the periphery of the cell (Fig.18).

The yolk nucleus consisted of even more complexes than in the 2-cell blastomeres and these complexes had changed their location, so that they were most abundant near the cell nucleus (Fig.15). They also had a more loosely arranged matrix that was partly split by a normal cytoplasm.

DISCUSSION

Copulation, ovulation, penetration of the spermatozoon into the ovum, and the cleavages of the zygotes are interrelated within rather fixed intervals (for ref., see Bronson et al.1966, Snell and Stevens 1966). The animals used in this study showed a well synchronized series of events, similar to the developmental pattern of other mouse strains (see Table I).

The fixation technique used resulted in a larger diameter of the ovum (88μ) than is commonly observed after various fixation techniques, (54 to 78μ)(Sobotta 1895, Kirkham 1907, Lams and Doorme 1907, Long and Mark 1911) or in culture media, (78 to 81μ, Lewis and Wright 1935, Brinster 1967b). The zona pellucida was somewhat thicker, 3.5μ, than obtained with other fixation techniques (1.2 to 2.0μ, Sobotta 1895, Long and Mark 1911) but thinner than those in culture media (5 to 10μ, Lewis and Wright 1935, Loewenstein and Cohen 1964). After fertilization there is normally a decrease of the cytoplasmic volume and a widening of the zona pellucida, so that a wide perivitelline space is formed (Lewis and Wright 1935). However, in the present material no widening of the zona pellucida was seen after the fertilization, but rather shrinkage. An unchanged and narrow perivitelline space was seen.

This larger size of the ovum and narrow perivitelline space could indicate some swelling of the ovum which occurs in media with low osmotic pressure or a high concentration of potassium ions (Austin 1961, Elmiger

1966). Since the osmolality of the fixative (400 to 500 mOsm) is higher than that in media (270 mOsm) giving optimal zygote development in vitro (Brinster 1965a), there should be rather a shrinkage of the ovum, but the special conditions during fixation of ova lying in situ in a large amount of oviductal secretions may influence the size of the ovum.

Fixation with glutaraldehyde resulted in the characteristics for good ultrastructural preservation of the ovum and zygotes: intact membranes of the nuclear envelope, the plasma membrane and the Golgi cisternae; uniformly fine and granular nucleoplasm and cytoplasmic matrix without empty spaces; no swollen mitochondria, but with the intramitochondrial vacuole specific for mouse ova (Wischnitzer 1967a,b). Glutaraldehyde also preserves the zona pellucida in the blastocyst stage (Reinius 1967b), contrary to conventional fixatives (cf. Hall 1935).

Some disadvantages are incident to the fixation method used. Firstly, the stage of the ovum cannot be determined before fixation. Secondly, the plane of the sections through the ova is entirely random, and thirdly, all ova in one oviduct cannot be studied. This was compensated for by sectioning many ova/zygotes of many animals also for light microscopy.

Quantitation in electron microscopy is rather difficult, since usually only a moderate number of plates are available, covering a minor part of whole cells. Further, the number of minor components, for instance ribosomes and glycogen granules, is dependent upon the thickness of the section and a changing affinity for the stain. Since the particular components vary in number and the volume of the ovum or zygote is not constant, the measurements on parts of cells might not be representative of the whole cell or the cell population. Thus, it is only rather large quantitative and also qualitative changes in the cell, that with a reasonable amount of work can be recorded. In this study no specific quantitative analyses were made, and the disadvantages mentioned were taken into consideration when describing, e.g., the increase of the ribosomes of the later zygotes.

Interpretations of qualitative and quantitative ultrastructural changes and their application to functional processes may be difficult, but the ultrastructure can often give information on a functional process. Correlated to other experimental results, it will provide confirmation of a functional process. The ultrastructural results of this study are of specific interest, since the ova and zygotes are fixed and observed in in situ conditions.

The ultrastructure of the ovum and zygote in the mouse are, in the main, in accordance with that in the rat (Sotelo and Porter 1959, Odor 1960, Izquierdo and Vial 1962, Mazanec and Dvořák 1963, Mazanec 1965, Szollosi 1965a,b, 1966, 1967, Schlafke and Enders 1967). Results from the rat will therefore also be included in the following discussion. For further information on the ultrastructure of mammalian ova, see reviews by Hadek (1965) and Stegner (1967).

In the following discussion the different developmental stages of the zygote will be characterized with regard to the assumed protein and energy metabolic activities and related to the ultrastructure of the zygote. The protein metabolism will be discussed from the aspects of the contents, the absorption and the synthesis of proteins and the contents and the synthesis of RNA and ribosomes, since these are all factors involved in the protein metabolism and can be studied by electron microscopy. Similarly, the energy metabolism will be discussed as regards stored and absorbed energy rich substances, and the oxidative activity. Further, the structural relationship between the zygote and the epithelium will be considered in order to elucidate the interaction of the zygote and the oviduct, and the significance of the oviductal secretion.

Three developmental stages are distinguished: 1)the unfertilized and the pronuclear ova in the ampulla, 2)the 2-cell zygote in the isthmus, and 3)the 5- to 8-cell zygote in the junctura.

Unfertilized and pronuclear Ova

Protein metabolism The protein concentration is similar to other tissue cells (Loewenstein and Cohan 1964). Little exogenous protein is absorbed by the ovum (Glass 1963, Mintz 1964, Monesi and Salfi 1967). There is probably a need for exogenous protein but it is not known quantitatively (cf. Whittingham and Biggers 1967). Protein synthesis is low or absent (Austin 1961, Mintz 1964, Brinster 1967c, Monesi and Salfi 1967, Hafez and Sugawara 1969), and the potentialities for synthesis are almost lacking, since the RNA concentration and synthesis are also very low (Braden and Austin 1953, Flax 1953, Austin and Bishop 1959, Mintz 1964, Monesi and Salfi 1967).

Electron microscopy also indicates low protein synthesis, since the nucleoli lack the granular part that plays a role in the RNA synthesis (Bernhard and Granboulan 1968, Hay 1968) and the ribosomes as well as

the granular endoplasmic membranes are few in the cytoplasm (Yamada et al. 1957, Sotelo and Porter 1959, Enders and Schlafke 1965, Mazanec 1965, Szollosi 1966, Schlafke and Enders 1967, and present results) (Figs.7 and 8).

The fibrous structures observed on electron microscopy probably constitute a large part of the protein content of the ovum and have been designated yolk (Szollosi 1965a, Schlafke and Enders 1967)(Figs.2,5 and 8). According to some authors they constitute ribosomal aggregates (Mazanec 1965, Weakly 1968), but this is not consistent with the RNA content (see references above). No ultrastructural signs of protein absorption such as those described fot the oocyte of the mosquito were observed (Roth and Porter 1964).

Energy metabolism. The energy metabolism occurs over the tricarboxylic acid cycle, mainly by the absorption of exogenous puruvate (Biggers et al. 1967, Whittingham and Biggers 1967). Stored lipids and carbohydrates may be of minor significance (Braden 1959, Loewenstein and Cohen 1964, Thomson and Brinster 1966, Stern and Biggers 1968, Hafez and Suguwara 1969). The oxidative activity seems to be low (Auerbach and Brinster 1967, Mills and Brinster 1967, Hafez and Sugawara 1969), indicating low energy metabolic activity.

Electron microscopy has revealed probable yolk components, i.e.fibrous structures and lipid granules, that may be important also as energy sources (Fig.8). A low oxidative activity of the ovum is indicated by the structure of the mitochondria with few and peripherally located cristae (cf. Fawcett 1966)(Fig.8). Mitochondria of a similar type have been observed in the mouse oocytes and rat ova (Yamada et al. 1957, Schlafke and Enders 1967), and also in mouse ova presented in a preliminary report (Sackler 1968).

Relation of the ovum to the oviduct. The ova were located in the ampulla, which was then distended by fluid, partly of epithelial origin (Reinius 1969a). Up to 10hr after the penetration the pronuclear ova were still surrounded by loosely arranged follicle cells.

Substances secreted from the epithelial cells have therefore to pass the follicle cells with their matrix, the zona pellucida, and the plasma membrane in order to reach the ova. However, the follicle cells and the zona do not seem to have any specific significance for the absorption and development of the ovum (Austin and Lovelock 1958, Whittingham 1968).

The type of secretion in the ampulla seems to be mainly proteinaceous (Reinius 1969a). This proteinaceaous secretion may constitute a certain source of nitrogen for the ovum (Whittingham and Biggers 1967, Whittingham 1968). The impression is obtained, however, that more proteinaceous material is secreted from the epithelium than is utilized by the ova, since their uptake of exogenous protein seems to be low (see discussion on protein metabolism). Other possible tasks of the proteinaceous ampullary secretion are to provide factors necessary for fertilization (cf. Brinster and Biggers 1965, Pavlok 1967, 1968), and for denudation of the ova.

Energy sources such as pyruvate cannot be observed by electron microscopy, although this should be secreted in the ampulla (Biggers et al. 1967, Wales and Whittingham 1967). Neither has glycogen been noted in the epithelium (Reinius 1969a).

Two - cell Zygote

Protein metabolism. The protein metabolism of the 2-cell zygote does not differ significantly from that of the ovum (Flax 1953, Austin 1961, Mintz 1964, Brinster 1967c, Monesi and Salfi 1967), although some new information is available: amino or acids equivalent to mouse serum, bovine serum or bovine serum albumin or the specific amino acid glutathione are essential for development in vitro, methionine is not absorbed in vivo, serum proteins are transferred from the oviductal epithelium to the zygotes in vivo, and non-nucleolar RNA synthesis of the nucleus has increased (Greenwald and Everett 1959, Glass 1963, Mintz 1964, Brinster 1965c, 1969, Weitlauf and Greenwald 1965, 1967).

Electron microscopy of the 2-cell zygote of the mouse indicates an unchanged protein synthesis compared to that of the ovum, since there is no alteration in the structure of the nucleoli and in the number of ribosomes (cf. rat, Sotelo and Porter 1959, Mazanec 1965, Szollosi 1966, Schlafke and Enders 1967). The increased nuclear RNA may be expressed as the increased number of granules measuring 100 to 150Å and 300 to 400Å in the nucleoplasm (Swift 1963)(Fig.11). Intranuclear annulate lamellae have been described earlier in invertebrate oocytes and embryos, in a pronuclear embryo and in early mouse zygotes (Merriam 1959, Hsu 1963, Kessel 1964, 1965, Zamboni et al. 1966, Hillman et al. 1967, Calarco and Brown 1968, Everingham 1968a,b).

The appearance IAL may indicate a change in, e.g., genetic information between the nucleus and the cytoplasm (Hsu 1963, Kessel 1965).

Energy metabolism. The energy substrates of the 2-cell zygote have probably not changed (Brinster 1965b, 1969, Brinster and Thomson 1966, Mills and Brinster 1967). However, the glycogen content has significantly increased (Thomson and Brinster 1966, Stern and Biggers 1968).

Electron microscopy has revealed no significant changes in the structures concerned with energy metabolism, compared with the ovum, and the findings are in accordance with those in in vitro studies.

Relation of the 2-cell zygote to the oviduct. The 2-cell zygotes lie in the middle loops of the isthmus, which are always distended by fluid. The zygotes can, however, come into direct contact with the epithelial wall, but only for short periods, since they are continuously being moved by muscular contractions of the oviduct (Burdick et al. 1942, Humphrey 1968). The secretion of the isthmus contains carbohydrates: only in the loops of the isthmus adjacent to the ampulla is proteinaceous material supposed to be secreted (Reinius 1969a). This proteinaceous secretion may constitute the necessary source of amino acids (Brinster 1965c, 1969). The glycogen known to increase in the 2-cell sygotes (Thomson and Brinster 1966, Stern and Biggers 1968) may correspond to the presence of a carbohydrate rich secretion in the isthmus.

Five to Eight Cell Zygote

Protein metabolism. The protein synthesis has increased significantly (Mintz 1964, Izguierdo and Roblero 1965). This is further indicated by an increase of nucleolar and cytoplasmic RNA (Flax 1953, Mintz 1964). However, according to Monesi and Salfi (1967) an increased protein synthesis and RNA content are not significant. However, since the protein content does not increase, a net synthesis may not ensue (Flax 1953, Austin 1961, Mintz 1964, Brinster 1967c). The need of exogenous protein has disappeared (Brinster 1965c).

The electron microscopical results, namely the development of a nucleolonema containing granules of the ribosomal type, and the increase in cytoplasmic ribosomes and endoplasmic cisternae with attached ribosomes (cf. Fawcett 1966, Sherbet and Lakshmi 1967, Bernhard and Granboulan 1968, Hay 1968) also indicate an increased protein synthesis (Figs. 15 to 18). The nucleolonema has been described for the rat as a "branching

mesh" or "netzartig Pheripheri" (Izquierdo and Vial 1962, Mazanec 1965) and is probably also partly correspondent to the perinucleolar structure designated the "nucleolus associated chromatine" (Austin 1953, Austin and Braden 1953, Flax 1953, Austin and Bishop 1959). Increased cytoplasmic ribosomes and granulated endoplasmic cisternae have been reported earlier for the mouse and the rat (Szollosi 1966, Hillman et al. 1967, Schlafke and Enders 1967). Crystalloid inclusions that many consist of partly proteins have also been described in morulae and blastocysts of the mouse in close association with the endoplasmic reticulum (Enders and Schlafke 1965, Calarco and Brown 1968, and present data) (Fig. 18). The close association of these crystalloid structures to the endoplasmic reticulum indicate that they should be synthesized there.

Energy metabolism. A change in energy metabolism has occurred, e.g. glucose and maleate are metabolized by the 5- to 8-cell zygote (Brinster 1965b, Brinster and Thomson 1966, Wales and Biggers 1968). The oxidative activity has increased significantly (Mills and Brinster 1967, Hafez and Sugawara 1969).

The ultrastructure did not provide any information on the carbohydrate metabolism. The change in the mitochondria, some of which have several cristae in the mouse and rat, is in accordance with the increased oxidative capacity (Fawcett 1966, Schlafke and Enders 1967, and the present data) (Fig. 18). However, no change in structure of the mitochondria of the mouse 8-cell stage has been reported (Sackler 1968).

An ultrastructural difference between the mouse and the rat that deserves attention is the distribution of the organelles. In the rat, cytoplasmic areas have developed which are devoid of organelles but contain fibrous elements (Izquierdo and Vial 1962, Mazanec 1965, Schlafke and Enders 1967). This does not occur in the mouse.

Relation of 5- to 8-cell zygote with the oviduct. The 5- to 8-cell zygotes lie in the junctura either extramurally or intramurally in very close contact with the epithelium (cf. Reinius 1969c). Almost no spaces are left for secretory products in the oviduct. No ultrastructural signs of an epithelial secretion are present, but signs of absorption are seen (Reinius 1969a). This is consistent with the reported lack of dependence of the zygote on exogenous protein (Brinster 1965c, Brinster and Thomson 1966). Information on carbohydrate secretion is not available, but it seems likely that carbohydrates are not necessary for the relatively short time of

transport through the junctura, since the zygote is loaded with glycogen (Stern and Biggers 1968, Reinius 1969c). The close contact between the zygote and the epithelium might, however, facilitate substance exchange.

SUMMARY

Ova and zygotes in situ in the oviduct of the mouse were examined by light and electron microscopy after fixation by aortic perfusion with glutaraldehyde, postfixation in osmium tetroxide, and embedding in Epon.

The structure of the nucleolus and endoplasmic reticulum indicates that the protein synthesis of the ovum and the 2-cell zygote is low and in the 5- to 8-cell zygote it has increased. Fibrous elements, probably yolk, occur abundantly and may partly constitute stored protein. The specific protein which is essential for the development of the ovum and the 2-cell zygote, is probably secreted in the ampulla and part of the isthmus. The independence of the 5- to 8-cell zygote to exogenous protein corresponds to the lack of protein secretion in the junctura.

The structure of the mitochondria indicates that the oxidative capacity of the ovum and the 2-cell zygote is low, but that of the 5- to 8-cell zygote is increased. The frequent distribution of probable yolk and lipid granules indicates that stored energy compounds occur to a large extent in the ovum. The glycogen known to increase in the 2-cell zygote may correspond to the presence of a carbohydrate rich secretion in the isthmus.

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LEGENDS

Fig. 1. Unfertilized ovum in the ampulla, Day 1/05 hr. The ovum is surrounded by many layers of follicle cells. The first polar body is visible to the right, the second spindle in the lower part of the ovum. Complex of yolk nucleus (arrow); zona pellucida (Zp). Light microscopy x 390.

Fig. 2. Unfertilized ovum in the ampulla, Day 1/05 hr. Cortical region showing a complex of the yolk nucleus, where multivesicular bodies (Mb) and vesicles of various sizes and shapes are distinguishable. Perivitelline space (Ps); plasma membrane (Pm); cortical granules (Cg); fibrous elements (F). Electron microscopy x 25,000.

Fig. 3. Unfertilized ovum in the ampulla, Day 1/08 hr. The follicle cells to the right lack cytoplasmic projections into the zona pellucida (Zp). This membrane has a loose outer part and a homogeneous inner part. The chromosomes of the second maturation spindle lie in a spool-shaped area devoid of organelles. The plasma membrane over this area is smooth, closely pressed against the zona pellucida and has an electron dense inner borderline. Mitochondria (M); lipid granules (Lg). Electron microscopy x 4,000.

Fig. 4. Pronuclear ovum in the ampulla, Day 1/15 hr. Follicle cells still surround the ovum. The zona pellucida is visible and is slightly elevated over the polar body. The two pronuclei - the smaller above is the female, the larger below the male - have one and three dark stained nucleoli, respectively. In the peripheri at the lower and the left part of the ovum two complexes of the yolk nucleus are visible. The granular part of the cytoplasm, consisting of mainly mitochondria and lipid granules, is concentrated around the pronuclei. Light microscopy x 860.

Fig. 5. Pronuclear ovum in the ampulla, Day 1/17 hr. A yolk nucleus complex is seen. Two central electron dense structures are surrounded by a thin area of less electron dense cytoplasm and around this area a large mass of mainly small vesicles and multivesicular bodies are observed. Perivitelline space (Ps); plasma membrane (Pm); fibrous elements (F). Electron microscopy x 23,000

Fig. 6. Pronuclear ovum in the ampulla, Day 1/16 hr. Detail of nuclear region. Perinuclear lacunae are formed between the two nuclear membranes (Nm) containing granules (arrows). Nucleus (N). Electron microscopy x 40,000.

Fig. 7. Pronuclear ovum in the ampulla, Day 1/15 hr. Detail of pronucleus with nucleolus (No) that stains homogeneously dark. To the left a group of granules, each granule measuring about 300 to 400 Å in diameter. Electron microscopy x 27,000.
Insert figure. Matrix of nucleolus with a fine fibrillar and/or granular appearance. Electron microscopy x 60,000.

Fig. 8. Pronuclear ovum in the ampulla, Day 1/15 hr. Cytoplasm containing mitochondria (M), that almost lack cristae and appear vacuolized. Golgi complex (G); vesicles (V), probably part of the endoplasmic reticulum; fibrous elements (F); lipid granules (Lg). Electron microscopy x 18,000.

Fig. 9. Pronuclear ovum in the ampulla, Day 1/16 hr. Part of a spermatozoon tail in the cytoplasm. Mitochondria (M) of the zygote with vacuoles; mitochondria (Ms) of probable spermatozoon origin. Electron microscopy x 25,000.

Fig. 10. 2-cell zygote in the isthmus, Day 2/16 hr. The zona pellucida is shaped according to the form of the two blastomeres, of which the plasma membranes are visible. The nuclei with nucleoli are located in the centres of each blastomere Granular components are homogeneously distributed; the dark granules are mainly mitochondria, the light are lipid. A small part of another zygote is seen to the left and the epithelium of the isthmus in the upper and lower parts of the picture. Light microscopy x 860.

Fig. 11. 2-cell zygote in the isthmus, Day 2/16 hr. Part of nucleus: Nuclear membranes (Nm), with pore-like structures (➧); intranuclear annulate lamellae (IAL) with pore-like structures (➧); nucleolus (No); granules with a diameter of 300 to 400 Å (300), and granules with a diameter of 100 to 150 Å (100);fibrillar condensation (Fc). Electron microscopy x 30,000.

Fig. 12. 2-cell zygote in the isthmus, Day 2/16 hr. The adjoining surface membranes lie at a distance of 0.1 to 0.2 μ apart, but the microvilli and broad cytoplasmic projections often intermingle or lie in direct contact with the apposing surface. Mitochondria (M); Golgi complex (G); vesicles (V), probably of the endoplasmic reticulum; complex of the yolk nucleus (Cy); fibrous elements (F). Electron microscopy x 28,000.

Fig. 13. 8-cell zygotes in the junctura, Day 3/17 hr. The zona pellucida surrounds the blastomeres, two of which have nuclei sectioned through the nucleoli. Dark granules are mainly mitochondria, the light are lipid granules. The epithelium is seen in the upper and lower parts of the picture. Light microscopy x 850.

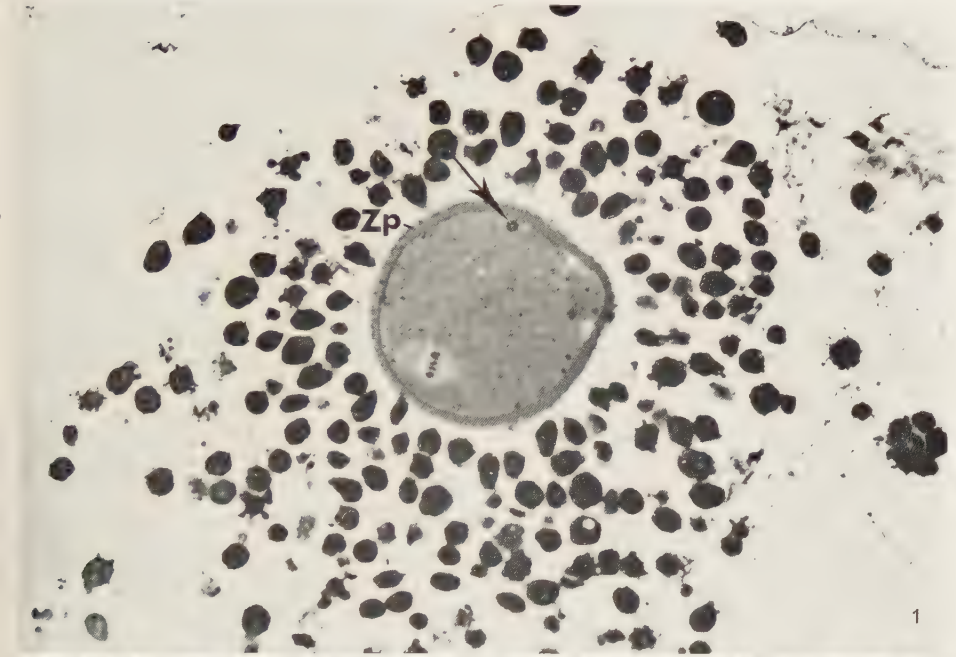
Fig. 14. 6-cell zygote in the junctura, Day 3/17 hr. Epithelium is seen in the lower part of the figure. Zona pellucida (Zp); nucleus (N); nucleolus (No); complex of the yolk nucleus (Cy). Electron microscopy x 3,000.

Fig. 15. 8-cell zygote in the junctura, Day 3/16 hr. The spherical nucleus has one nucleolus (No) with a central homogeneous electron dense structure and an outer net-like periphery, the nucleolonema. Arrows point to structures composed of granules 300 to 400 Å in diameter. Nuclear membranes (Nm); plasma membrane (Pm); mitochondria (M); crystalline structures (Cs); complex of the yolk nucleus (Cy). Electron microscopy x 8,000.

Fig. 16. 6-cell zygote in the junctura, Day 3/17 hr. Part of the nucleus is seen to the left, the cytoplasm to the right. The nuclear membranes (Nm) have several nuclear pores (➧). An intranuclear annulate lamella (IAL) also demonstrates pores (➧). The IAL is in contact with both nucleolar structure (No) and the nuclear membrane. Electron microscopy x 30,000.

Fig. 17. 8-cell zygote in the junctura, Day 3/17 hr. Part of the nucleolus with the homogeneous fine fibrillar inner structure and the peripheral strands partly composed of granules and/or fibrillar structures about 100 to 150 Å in diameter (arrows). Electron microscopy x 60,000.

Fig. 18. 8-cell zygote in the junctura, Day 3/17 hr. Part of the nucleus is seen in the upper right corner; nuclear membranes (Nm). The cytoplasm contains a mitochondrion (M) with some transverse cristae; ribosomes (arrows); crystalline structure (Cs) surrounded by a cisternal structure with attached ribosomes; fibrous elements (F); myelin-like figure (Mf). Electron microscopy x 34,000.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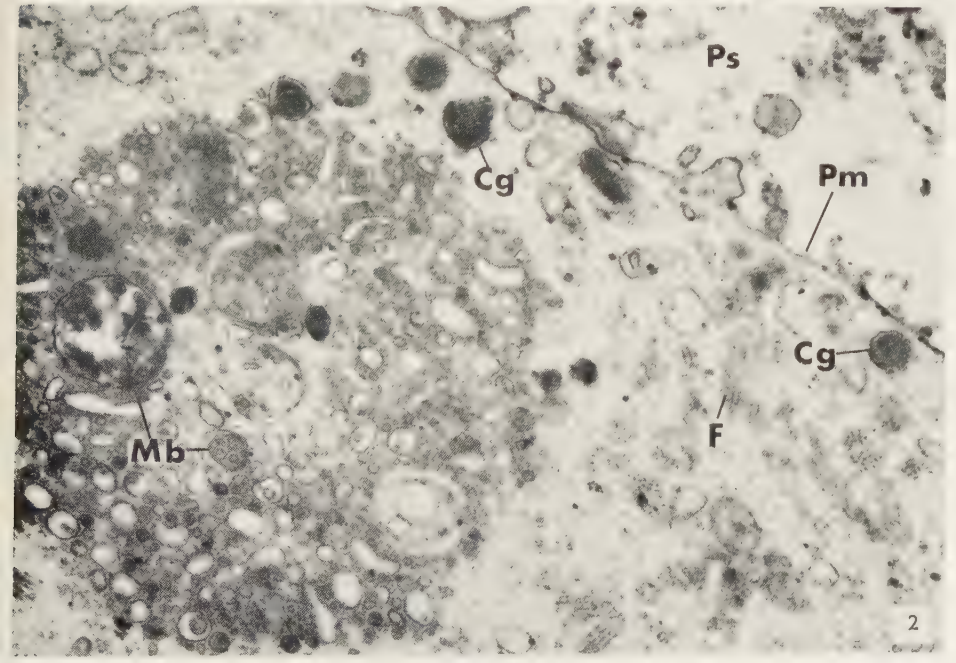
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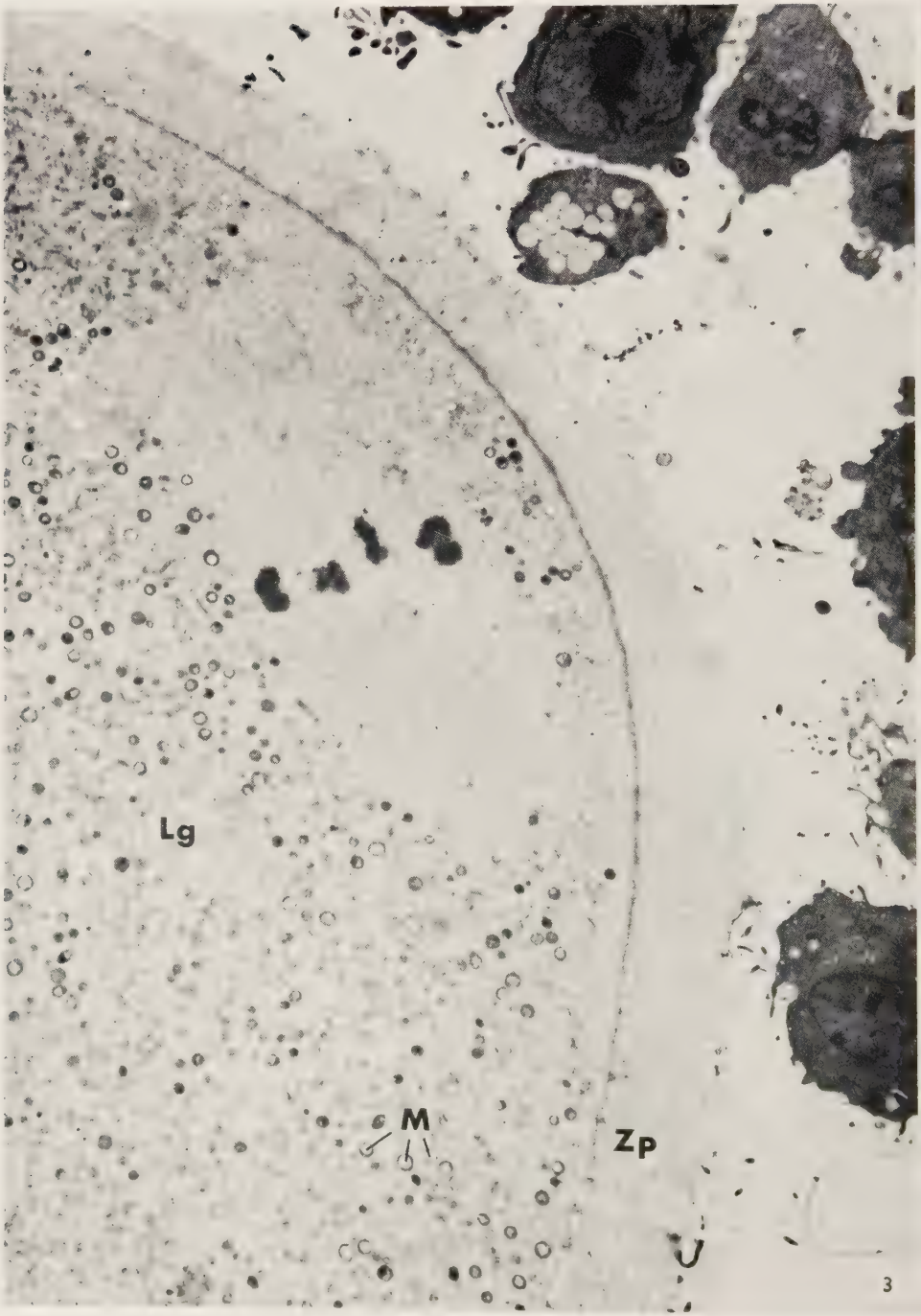

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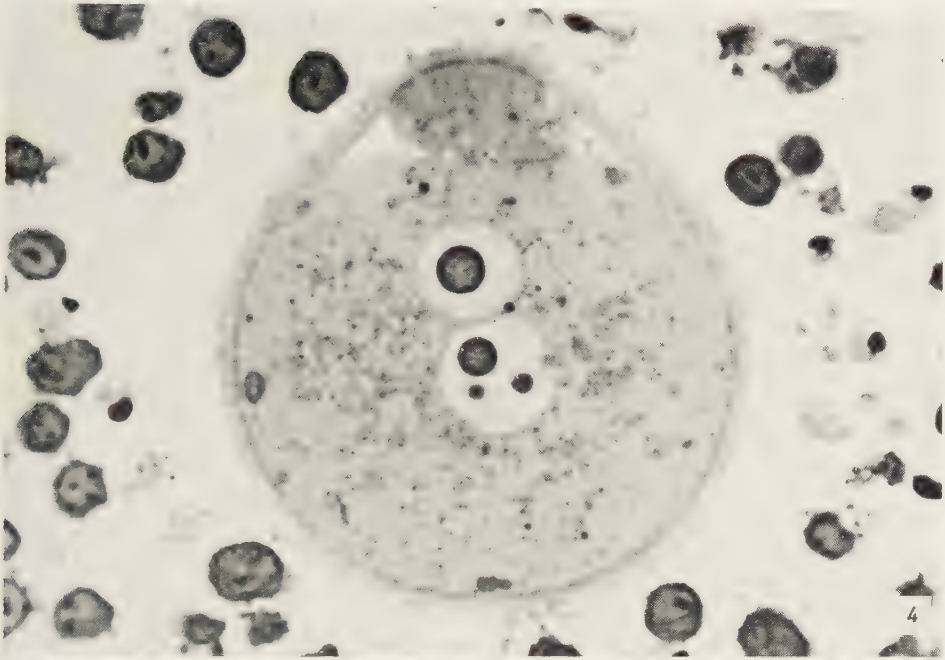

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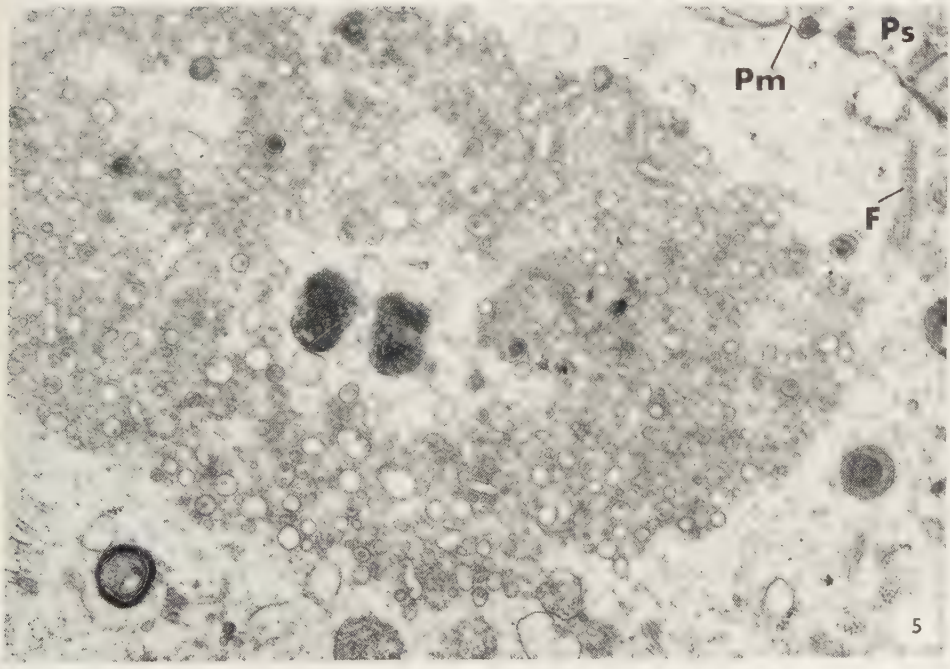

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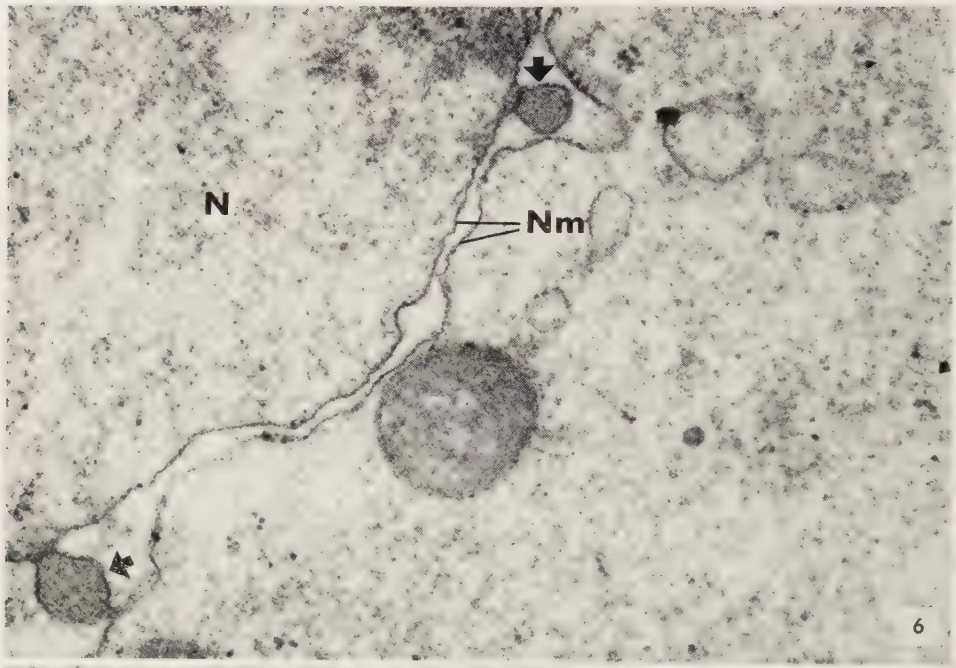
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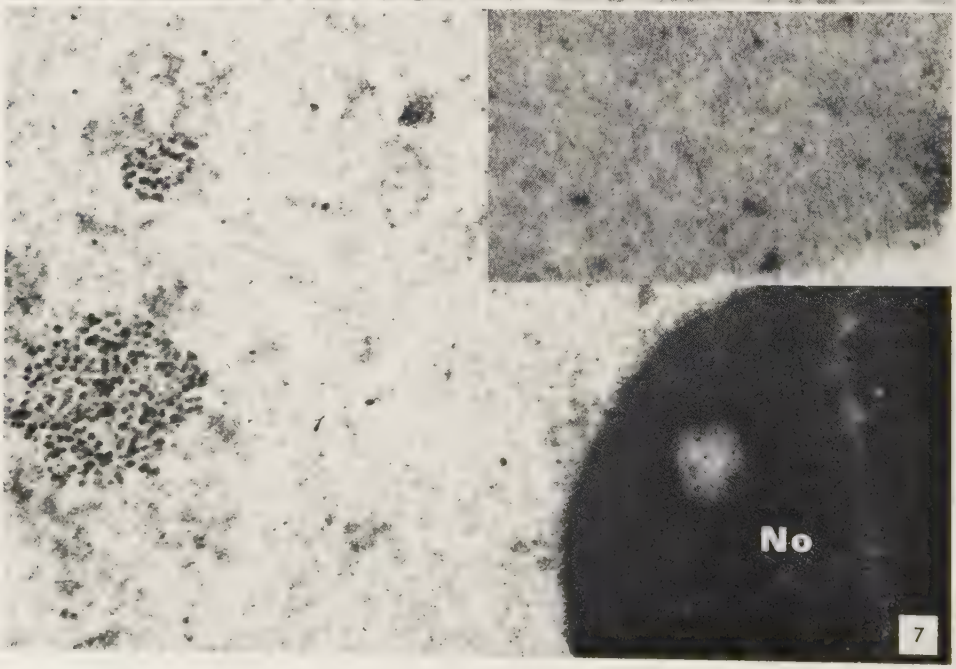
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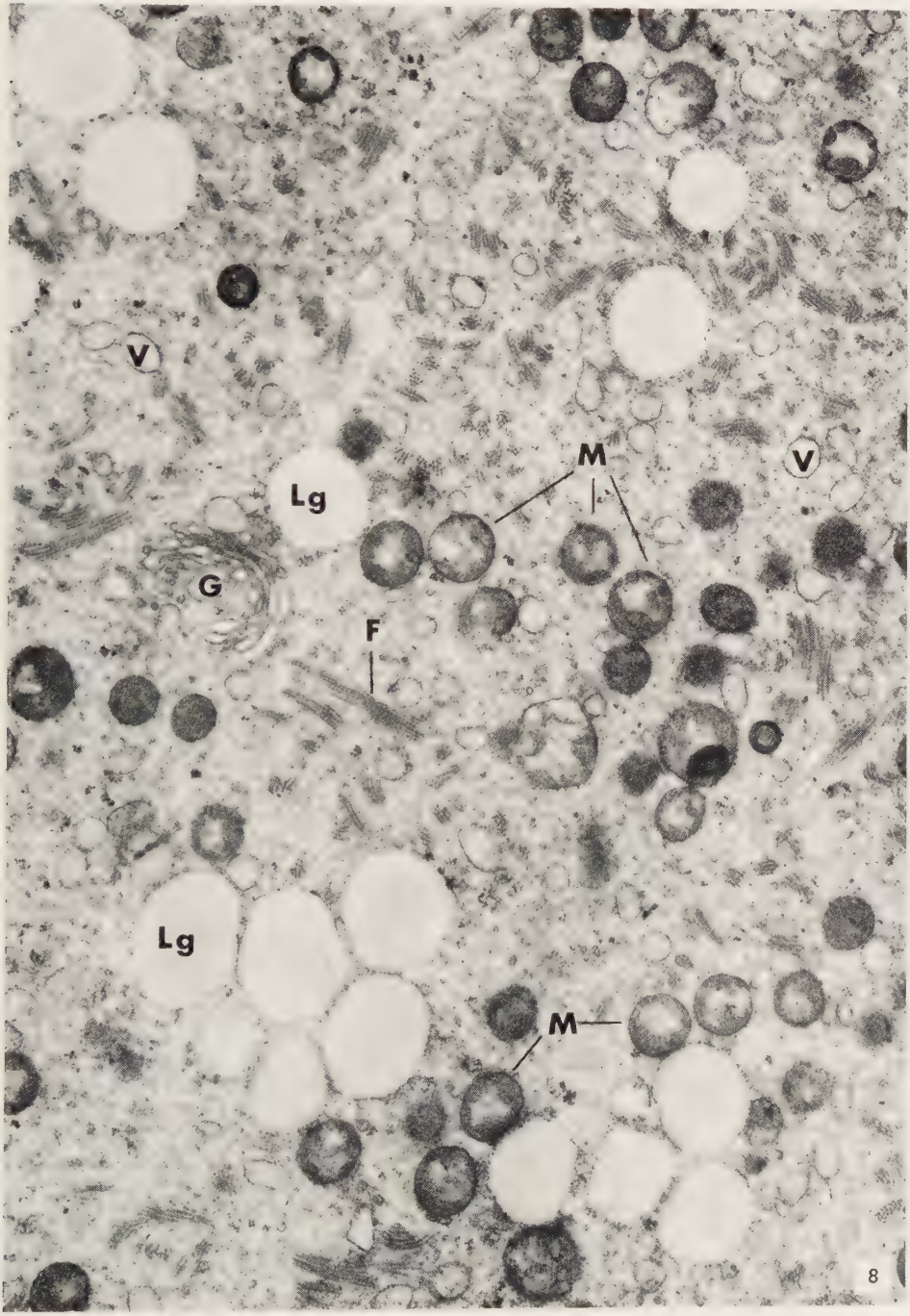
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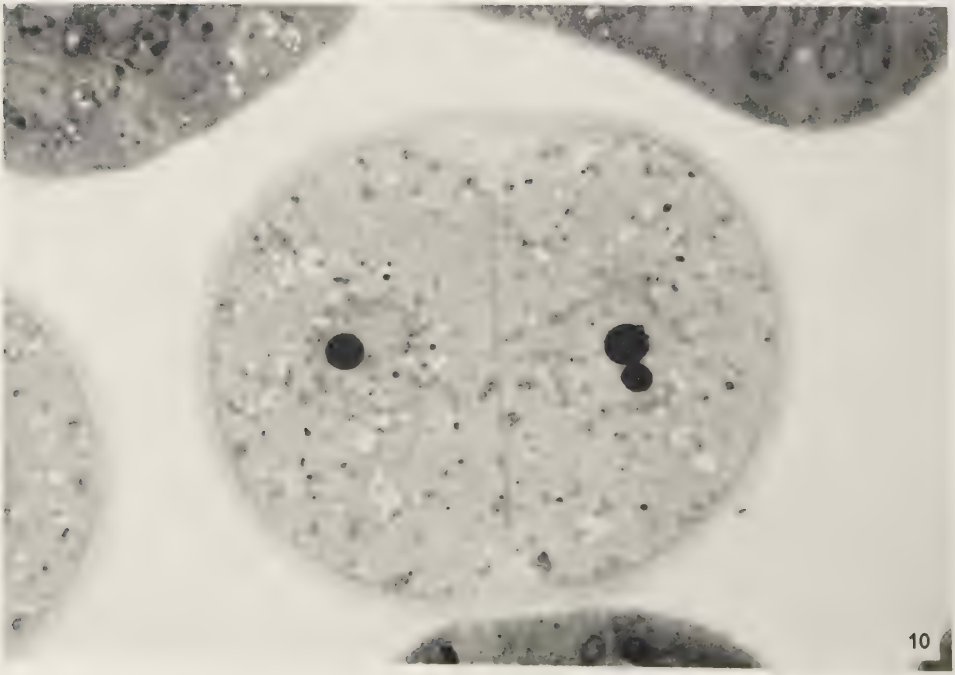
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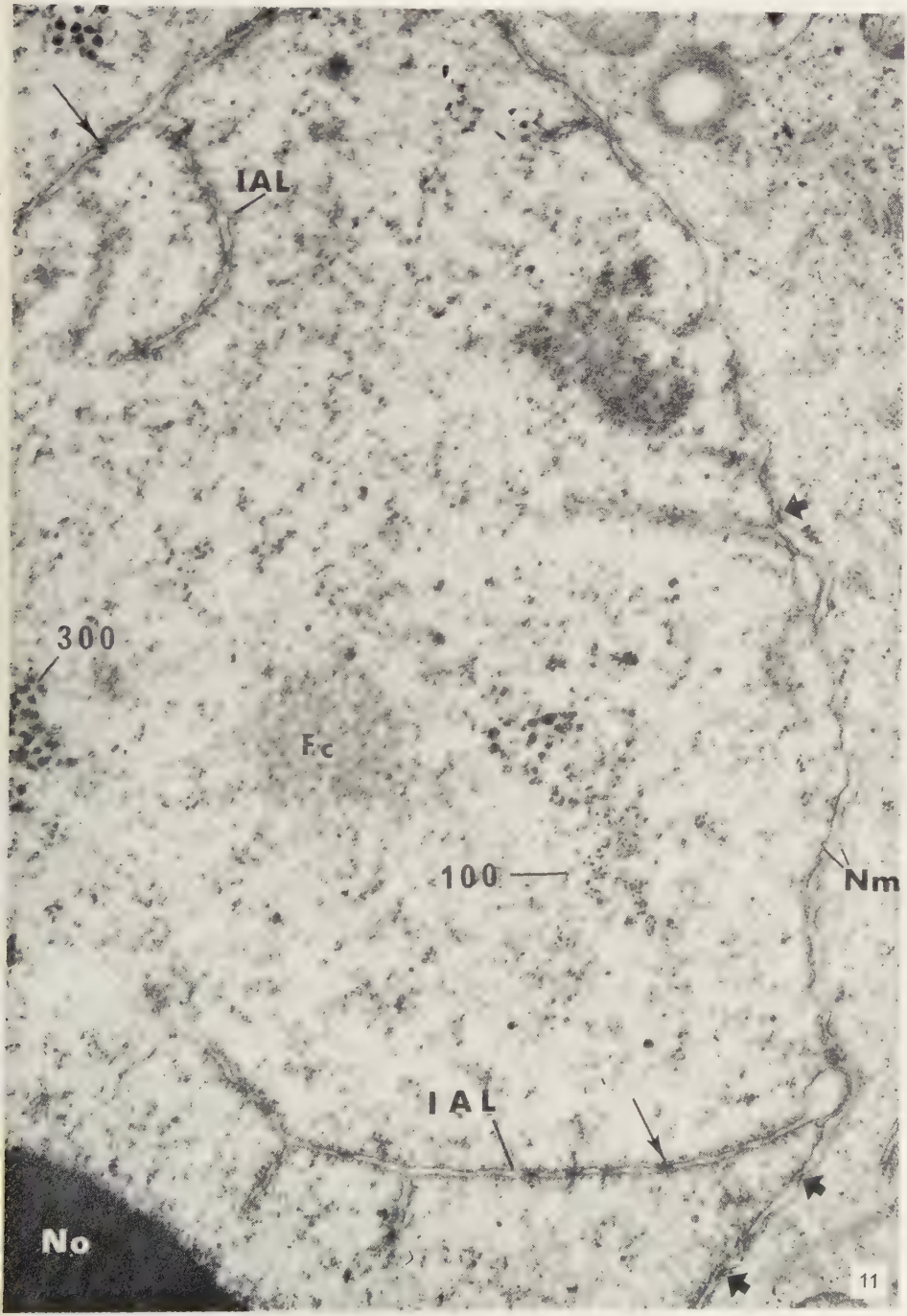
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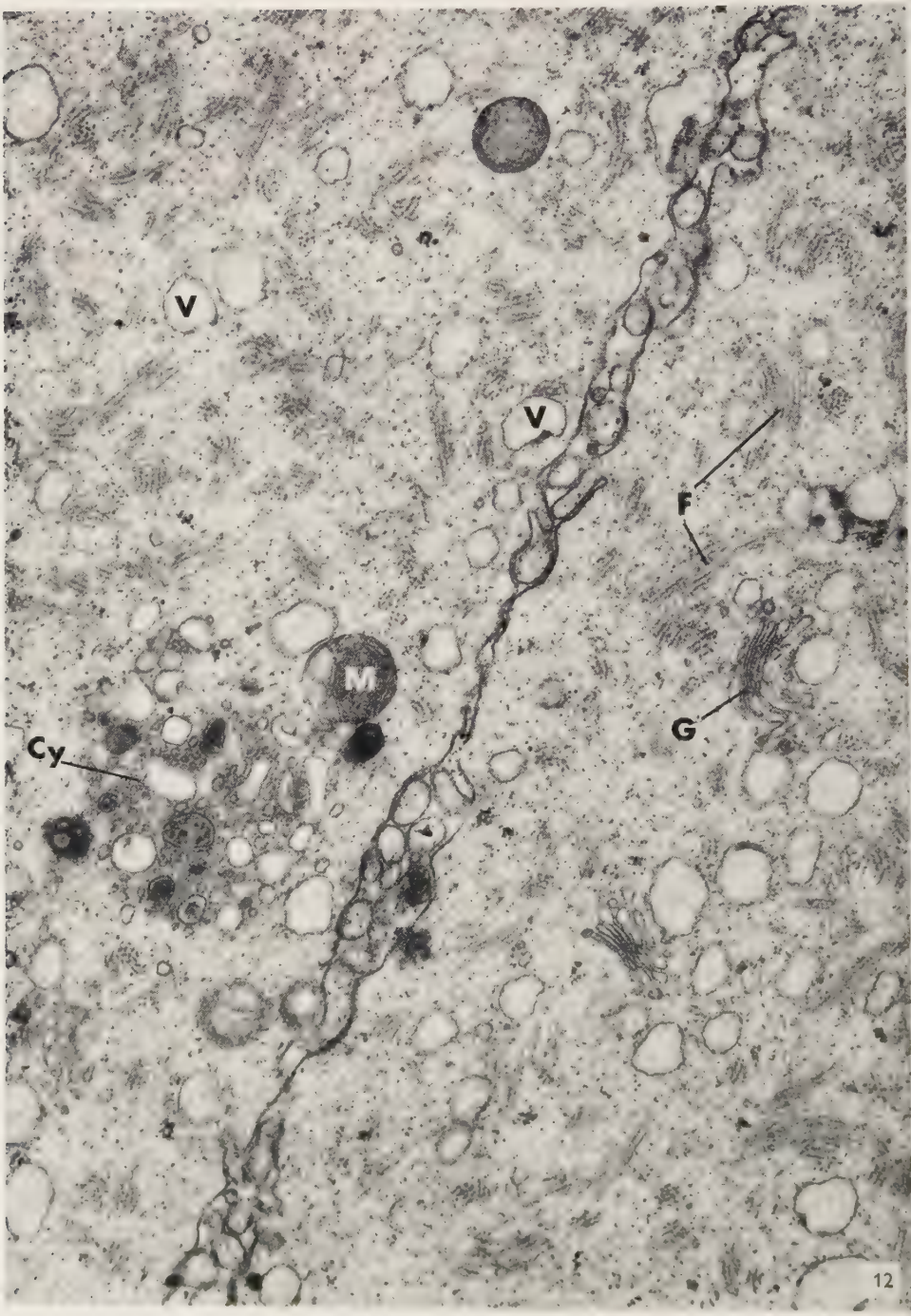
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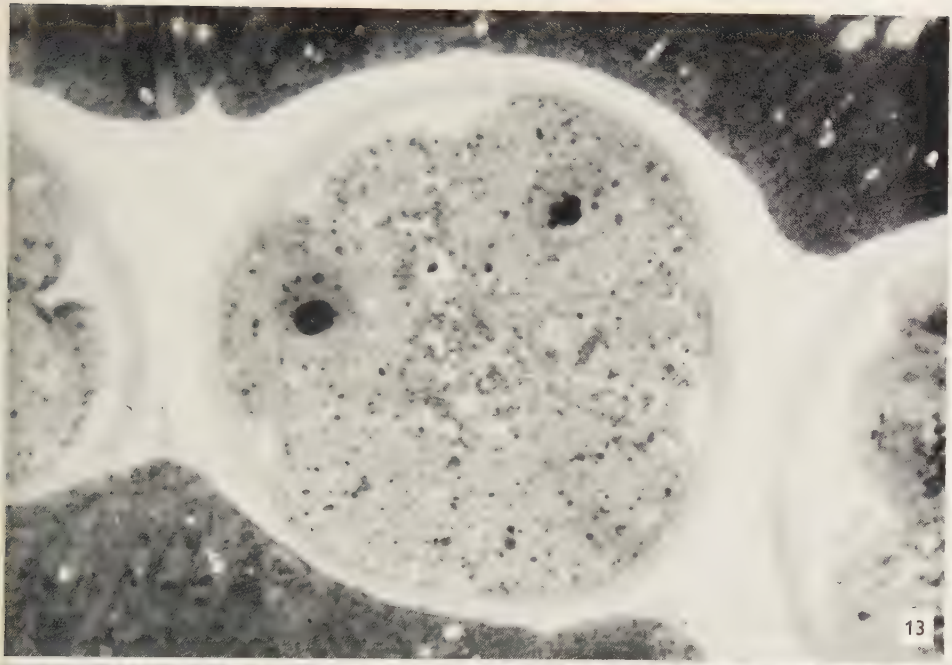
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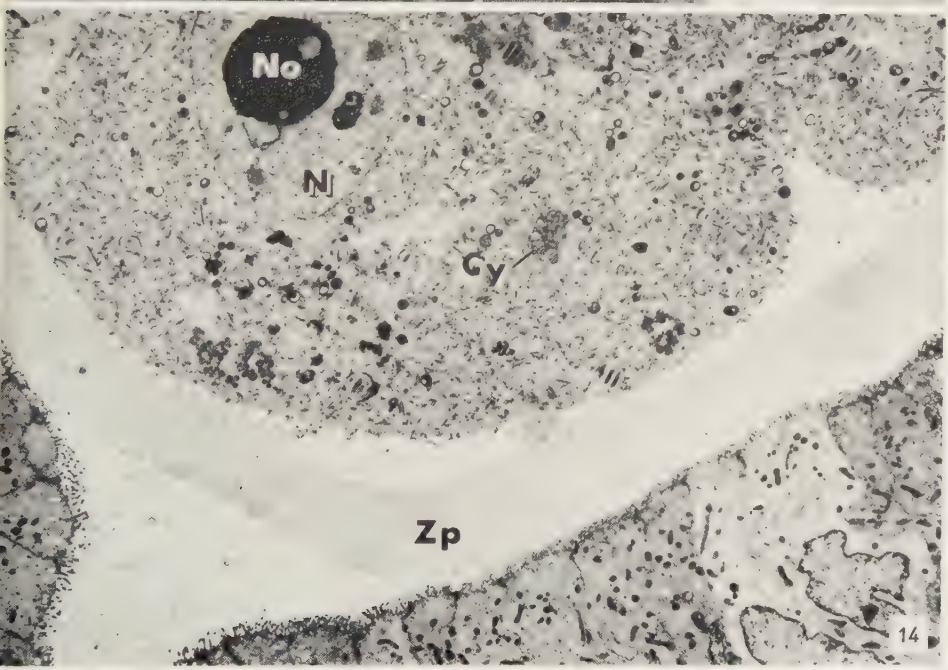
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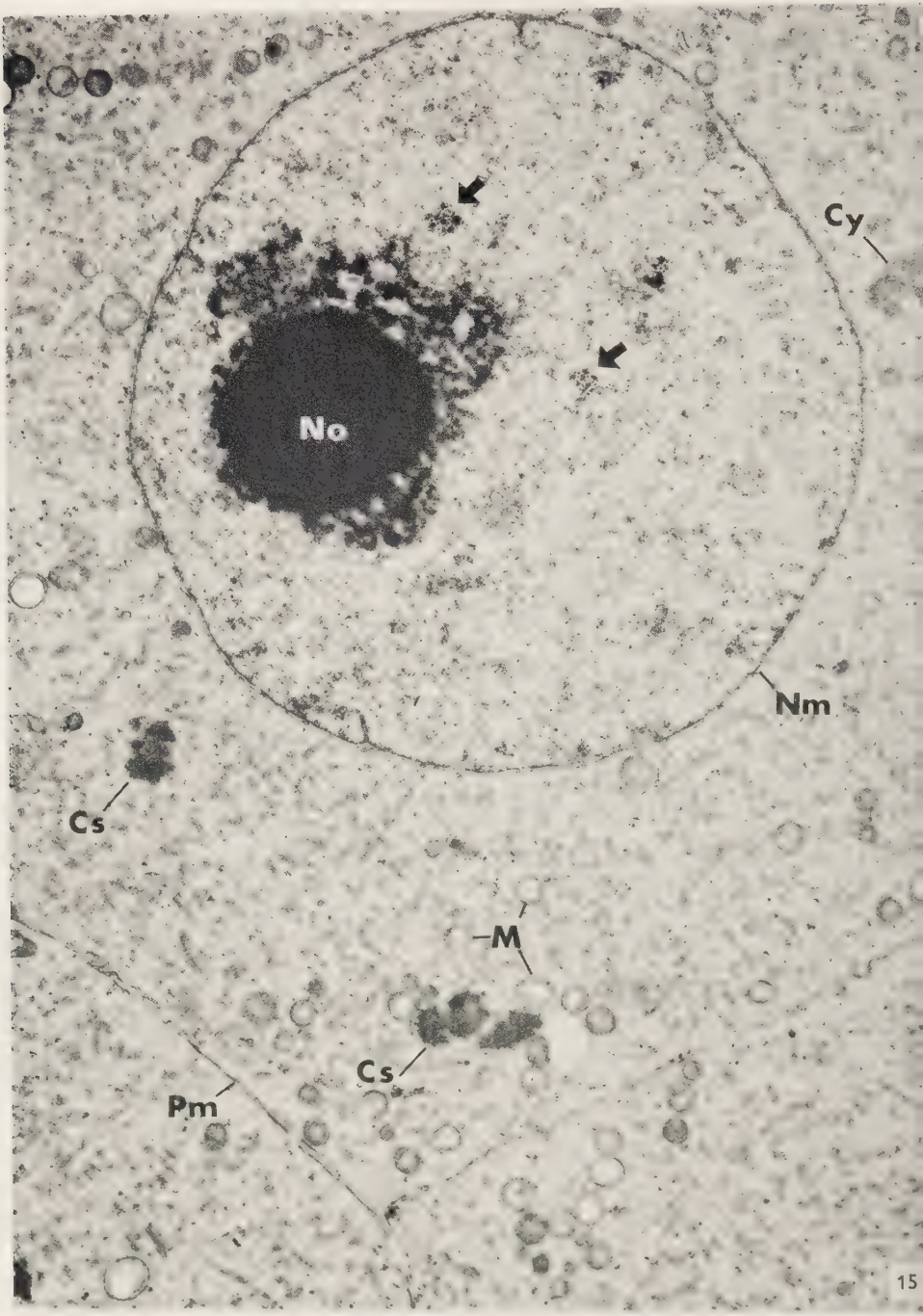
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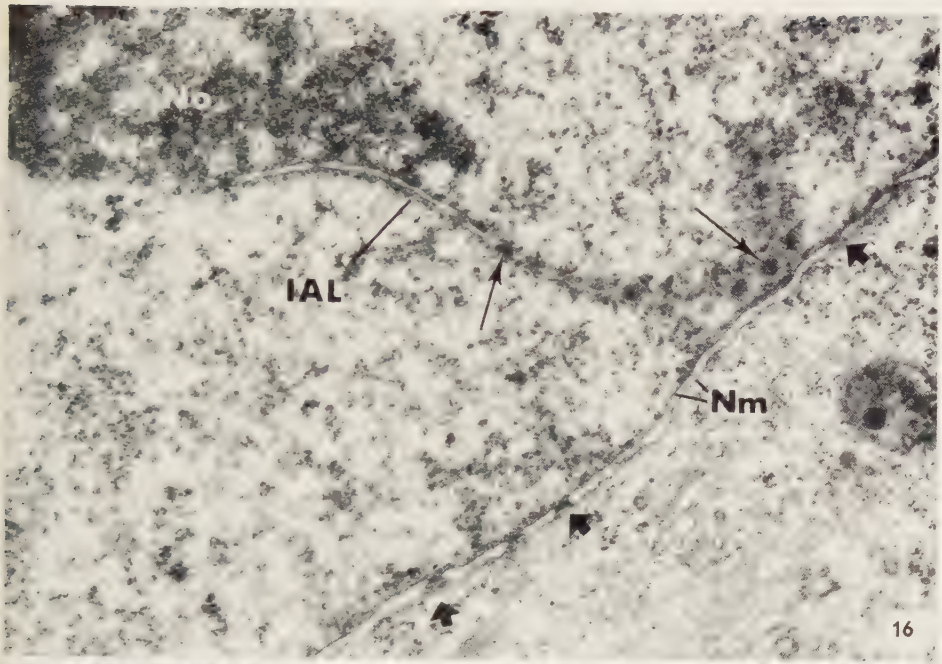

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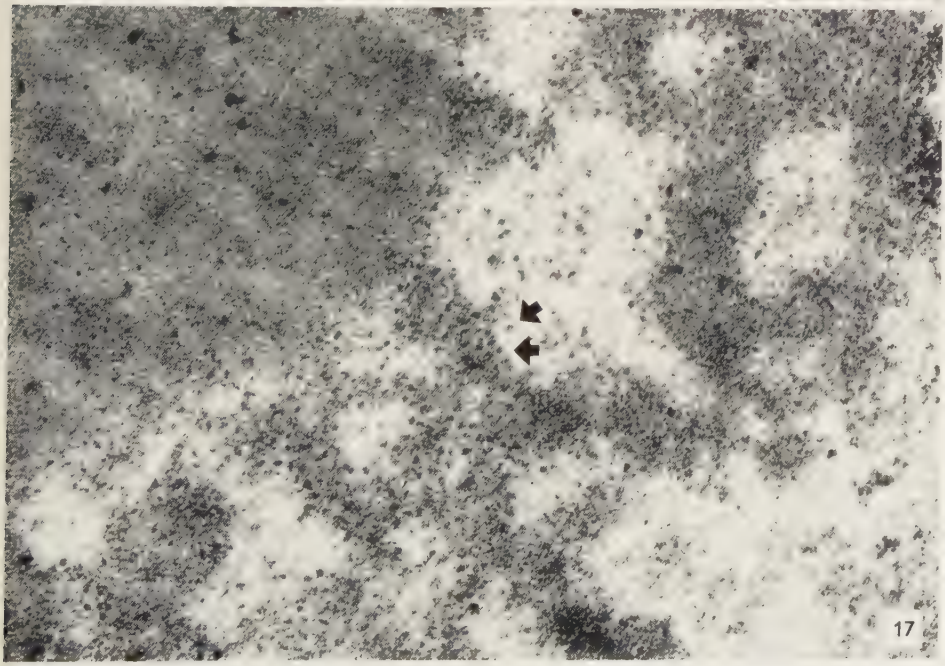

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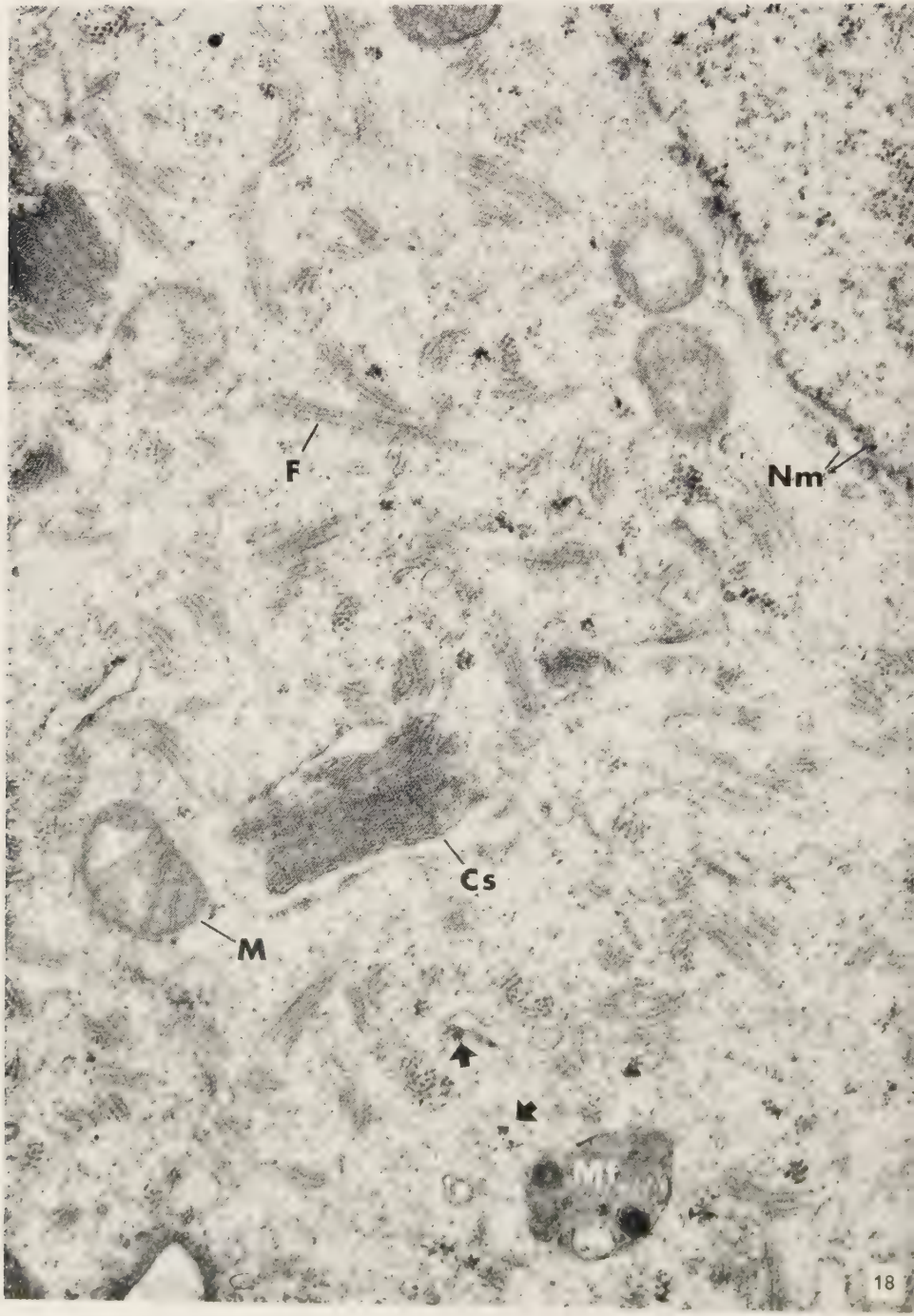
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